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THE SIGNIFICANCE OF NITROGEN ESTIMATIONS IN THE BLOOD IN ARTERIAL AND KIDNEY DISEASE*

BY I. JESSE LEVY, PH.D., M.D., NEW YORK CITY

IT is evident that various symptoms supposed to have their origin in kidney pathology are not due, directly at least, to disease of the kidney. Because of this fact, it seemed desirable to correlate the nitrogen estimations in the blood (antemortem) with the postmortem findings in the kidney.[1]

Before correlating any changes in the organ with the various nitrogen concentrations in the blood, it was found necessary to have a clearer idea of the pathologic processes common to the kidney. This problem presented itself to us some years ago, and for this reason, we became interested in the study of the kidney based on these requirements. In 1919 I[2] reported "A Pathological Classification of Nephritis Based Upon Clinical Findings," in which great stress was laid upon the fallacy of such designations as "interstitial nephritis," "parenchymatous nephritis"; terms which were ambiguous in describing pathologic processes in the kidney. For a more sensible conception of renal pathology, it was noted that the interpretation of kidney changes in the light of previous clinical phenomena was an important factor, and this led to a slight modification of, or rather an addition to, the classification then given. In other words, it was found difficult, in studying the damage seen on a kidney slide, to ascertain from which radicle of the kidney the disease originated, (the importance of this is discussed elsewhere) without some knowledge of the clinical course of the disease. Other factors which aid in clarifying the primary source of any kidney damage are a thorough understanding of the associated pathology; i.e., the type of cardiac hypertrophy, the character of the arterial changes, the absence or presence of a definite source of infection, etc. Having ascertained the primary

*From the Strecker Memorial Laboratory, City Hospital, New York City.
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pathologic focus, it is important to undersand the course of the further development of the pathology. Several factors often made this a disturbing matter. Irrespective of the origin of the pathology, it was learned that these factors when at work produced kidney pictures of striking resemblance, and independent of which radicle of the kidney became first involved, the final stage in the development of the pathology may be very similar. This led to consideration of the nomenclature. Lack of ambiguity is an essential feature of any classification. The terms must imply clearly and definitely the several features of any one pathologic change. This can be done best by considering the kidney as a unit, in which we note the variation from a normal organ to one which is completely destroyed (the so-called "structureless kidney") the intervening stages presenting various degrees of "structural changes." We can speak, then, of the "structureless kidney" of a glomerulonephritis or of a "structureless kidney" in a precapillary sclerosis, etc. To this general conception of renal pathology must be added the idea of "normality" to the course of any pathologic process. This can be more clearly understood when it is stated that there is a tendency for one or more types of kidney disease to develop into structureless organs, whereas others tend to adhere to their normal structure or undergo only slight structural changes.

In studying the relation of clinical phenomena to the underlying pathology, certain facts are disregarded, and this is especially true in kidney pathology. A diseased organ necessarily does not directly give rise to any symptoms, and in their absence one must not always anticipate finding an organ free from pathologic change. Only certain types of structural changes, as far as the kidney is concerned, give rise to clinical phenomena, and the degree of destruction runs fairly parallel to the severity of the disease. Fully understanding, then, that structural changes could, under certain conditions, give rise to recognizable disease and that the same pathologic picture is seen often unaccompanied with clinical symptoms, the explanation was sought in a pathologic study of the kidney, with a close adherence to the clinical course of the disease. It was learned that structural changes in the kidney, depending upon from which radicle of the kidney the disease originated, gave rise to varied clinical pictures, and that each such type of pathology adhered fairly constantly to any one clinical type.

The development of kidney pathology based upon the involvement of any one of the kidney radicles, through the stages of structural changes and structureless kidney, can be roughly followed with the accompanying chart. The three primary radicles are the glomeruli, the vessels and the tubules. Three types of vascular disease are given—the precapillary type, decrescent arteriosclerosis, and the intermediary type. The first two can be clearly defined, the last includes a heterogeneous series of cases which have a striking pathologic and clinical similarity.

GLOMERULAR DISEASE

Involvement of the glomerulus gives rise to true kidney disease, and there are two large groups, the (1) primary—with no demonstrable infec-

tious focus in the body, and (2) secondary—those following some infectious focus, usually an infectious endocarditis. At autopsy the primary types usually are seen as structureless kidneys; individuals who have died of their kidney lesion. The secondary types are seen occasionally as structureless organs in individuals who have suffered with a healed endocardial lesion (Libman). Usually, however, the kidneys present only various grades of structural changes.

VASCULAR DISEASE

Vascular disease of the kidney is a part of the general arterial process. Only in the precapillary type can we clinically speak of renal involvement. In the other two types, the patient presents seldom, if ever, renal symptoms. This agrees fairly well with the pathologic course of the disease, in which there is a tendency, under certain unknown conditions, for the precapillary type to take on definite structural changes, or to develop into practically structureless kidneys. The slight structural changes noted in the other two types are seldom as striking or as marked.

TUBULAR DISEASE

Primary involvement of the tubules is a degenerative process. The interstitial types are the result of connective tissue replacement of the degenerated tubules, presenting kidneys with structural changes. Only when the glomeruli are the seat of amyloid disease does one meet with structureless kidneys in primary disease of the tubules. The primary interstitial nephrotic kidney is found without any known source of infection, toxic agent or irritation in the body. The secondary type is the result of a long standing empyema, a caseating tuberculosis, diabetes, eclampsia, etc.

Retention of nitrogen in the blood was considered at first to be synonymous with kidney disease. That this is not true became known in a short time from a clinical survey of the material. From the standpoint of the kidney, our present knowledge of nitrogen retentions in the blood can be summarized in the following four groups:

1. Retention present in undoubted clinical cases of kidney disease.
2. Present in doubtful kidney cases.
3. Present in cases with no apparent kidney involvement.
4. Absent in certain types of renal disease.

Though the above seems to be true clinically, it is difficult, well-nigh impossible, to translate it into pathologic terms. None of these groups falls cleanly into any pathologic classification popularly used. Under such conditions of course, no one could hope to utilize nitrogen retention in the blood with the view of making a pathologic diagnosis. The conception of kidney pathology as outlined above, holds out more than a hope for such a possibility.

The nitrogen concentration in the blood materially arises in kidney and arterial disease just before death, with more or less variation, depending upon certain variable factors, irrespective of the pathology present in the

kidney. Because of this usual rise and its importance in diagnosis and prognosis, it is essential to consider this period independently. Nitrogen figures in the blood during this interval will be designated as "terminal." In this study, an arbitrary time of five days before death was chosen. All other nitrogen estimations fall into the "intercurrent period." Excepting as a therapeutic guide, slight variations in blood nitrogen figures have very little value. Even wide variations at times are difficult to analyze. For practical purposes, therefore, and for convenience of study, nitrogen retentions will be placed in three main groups: moderate, marked and excessive. The limits of each group have been chosen arbitrarily, namely:

1. Moderate retentions; all retentions below 40 mg. urea nitrogen or 80 mg. of nonprotein nitrogen per 100 c.c. of blood.

2. Marked retentions; all retentions below 100 mg. urea nitrogen or 125 mg. nonproteid nitrogen per 100 c.c. of blood.

3. Excessive retentions; all estimations above these figures. (The *normal* is placed at the high upper limit of 22 mg. urea nitrogen or 40 mg. nonproteid nitrogen per 100 c.c. of blood.)

From the conception of kidney pathology as outlined above, only those individuals in whom structureless kidneys are found at autopsy can be considered as dying of their kidney lesions. In other words, only the terminal stage of kidney pathology can give renal death. Kidneys with structural changes hold only a secondary position in the cause of death.

What, then, is the rôle of the structureless kidney in the nitrogen metabolism as manifested by gross changes in the blood? The three types of structureless kidney are the result either of glomerular disease, of a precapillary sclerosis with renal involvement, or of the interstitial nephrotic kidney with amyloid changes in the glomerulus.

STRUCTURELESS KIDNEY: END STAGE OF A CHRONIC GLOMERULITIS

It is apparent from a glance at the chart that there is a complete breaking down of the nitrogen metabolism in all the cases in both the terminal and intercurrent periods. The excessive retentions in the intercurrent period were present on an average for about twenty days before death. The noncontracted kidneys about forty days before the final outcome showed marked retentions, with only moderate retentions forty-five days before. At no time in the hospital were the blood figures normal. The contracted kidneys, however, showed a marked retention 210 days before, and a moderate retention 240 days before death.

All individuals, then, dying with this type of kidney give terminal excessive retentions in the blood, present on an average three weeks before the end. The record of a moderate retention only forty-five days before death, with a noncontracted kidney, might lead one to believe that the destruction of the kidney at this date should have given a higher retention. However, when one considers that the course of the disease is both very rapid and acute, and that the moderate retention may have been present some time before the patient came to the hospital, it seems fair to assume

		TUBULES			GLOMERULUS			BLOOD VESSELS				
		(A) Tubular Type (NORMAL 100%) (B) Interstitial Type (Amyloid Glomer)			Chronic Glomerulitis (A) Primary (B) Secondary			Pre-Capillary Type (A) Vascular Type NORMAL 100% (B) Renal Type			Intermediary Type Vascular Type	Decrescent Arteriosclerosis Vascular Type
		Structural Changes		Structureless	Structureless		Structural Changes	Structureless	Structural Changes		Structural Changes	Structural Changes
		Prim.	Second.		N.C.	C.		C.	C.	N.C.	C. & N.C.	C. & N.C.
TERMINAL	Ex		50%	100%	100%	100%	75%	60%	50%		15%	15%
	Mk						25%	40%	50%	50%	35%	70%
	Md	100%	50%							50%	15%	
	N										35%	15%
INTERCURRENT	Ex			100%	100% (20)	100% (20)		50% (15)				
	Mk				(40)	(210)	35% (10)	50% (16-160)	100% (24)	50% (8)		
	Md		100%		(45)	(240)		(90-180)	(90)	50% (26)	80% (8-120)	40% (30)
	N	100% (24-150)					65% (30-60)				20% (30-150)	60% (10-180)

Abbreviations: Ex, Excessive; Mk, marked; Md, moderate; N, normal; N.C., noncontracted; C, contracted.

The percentage figures indicate approximately the number of cases of the series falling in the various retention groups. The figures in parentheses with the percentage figures indicate the average number of days before death such retention was present. The figures in parentheses below the respective percentage figures indicate the average number of days before death blood figures were taken, and show the type of retention at this particular date.

that the pathologic process may be the cause of this retention. With the contracted kidney, as stated, there was a marked retention on an average about seven months before death, and moderate retention eight months before, and perhaps longer. Appreciating that the course of the disease is much slower in this type than in the previous one, the nitrogen retentions seem to coincide with the course of the pathology. We have evidence here that the structureless glomerular kidney may be a *direct cause* of a blood nitrogen disturbance. If, for reasons which will be discussed later, it may be assumed that it is not an immediate direct cause, it is certainly true that this type of pathology is always *associated* with nitrogen retentions in the blood; though the higher retentions are of extrarenal origin.

It is difficult to estimate at what point in the pathology we get disturbances in the nitrogen metabolism. A study of the figures in the glomerular kidneys with structural changes brings out some interesting facts. Seventy-five per cent of these cases died with excessive, and twenty-five per cent with marked terminal retentions, though during the intercurrent period there was only a slight disturbance in the blood, present in about thirty-five per cent of the cases, and normal figures in about sixty-five per cent of the cases. It is quite clear from this that the high terminal retentions are not the result of the kidney pathology, and that structural changes even in this type of kidney do not produce any gross nitrogen disturbance. It may be argued, however, that this series of cases consists of early and fresh kidney lesions, (death resulting from nonrenal organic pathology) and it may be assumed with fairness that the kidney lesion did not exist at the date the blood specimens were taken.

STRUCTURELESS KIDNEY, IN PRECAPILLARY SCLEROSIS

The term "structureless kidney" as applied to the final stage of renal disease in a precapillary sclerosis is used in a less strict sense than in the other types. This is necessarily so because the disease is a vascular one, and not renal. The destruction of the kidney tissue depends to a very great degree upon the severity of the vascular lesion. That other unknown factors are at work is discussed in another place.[1] The interpretation of the nitrogen derangement from the renal point of view is complicated by the various sources of disturbance caused by the vascular disease. Generally, however, precapillary sclerosis uncomplicated with structural changes in the kidney (the vascular type of the chart) or unassociated with objective evidence of cardiac decompensation, shows no disturbance of the nitrogen metabolism. The figures on the chart are 100 per cent normal. With the onset of the kidney involvement, however, there is usually evidence of cardiac embarrassment, and the nitrogen retentions must be considered from the point of view of the circulation, as well as the kidney.

Sixty per cent of individuals with structureless kidneys resulting from a precapillary sclerosis, die with excessive terminal retentions, the remaining forty per cent with marked retentions, though the histologic appearance of the specimens is very similar. This difference in the terminal retentions must

be the result of some other factor than the kidney. Records on the nitrogen figures in this series show that fifty per cent of the cases gave excessive intercurrent retentions, on an average fifteen days before death, the other half only marked retentions varying 16 to 160 days before. Even during this period, there can be very little change in pathology of the kidney, so we are forced to conclude that these differences in the degree of retention result from other factors than the renal pathology, even in the face of such definite kidney destruction, probably the final break-down of vascular disease. These same cases, three to six months previously, give nitrogen figures which fall into the moderate retention group. The question arises, are these moderate retentions of renal origin? The fact that when these patients present evidence of nitrogen derangement, there is already evidence of an embarrassment of the circulation, indicates that an extrarenal factor is the important one. I have never seen a case in which the circulatory disturbance did not dominate the picture. The individual suffering with precapillary sclerosis uncomplicated with renal change, has a circulation which is well maintained; the exodus is sudden, cerebral hemorrhage or coronary thrombosis. It appears that structural change in the kidney is a definite insult to the circulation, which brings about the circulatory decompensation, and with it a break in the nitrogen metabolism. It will be shown that the renal involvement, strictly speaking, may be the dominant, but is not the direct factor.

This idea is further emphasized by a study of the nitrogen figures in patients dying with structurally changed kidneys in the course of a precapillary sclerosis. It will be noted from a glance at the chart that these kidneys with far less destruction give terminal retentions very similar to those found in the structureless types. The intercurrent retentions (100 per cent marked retentions for the contracted kidney, on an average 24 days before death, with records of a moderate retention on an average of 90 days before the final outcome, and for the noncontracted organs 50 per cent marked retentions and 50 per cent moderate retentions) present a striking similarity to the above in the type of retentions, with the difference that they are of less severity, and present over a shorter period. The fact is not to be doubted that we have here two types of renal pathology, differing widely in their histologic appearance, but presenting during life similar disturbances in the nitrogen concentrations in the blood. A plausible explanation can be found in the circulatory break which is induced by the structural changes in the kidney.

Further evidence that localized structural changes in the kidney have no direct influence upon the gross changes of the nitrogen constituents of the blood, can be deduced from a study of the kidney in the so-called decrescent arteriosclerosis. The chart shows that in the terminal period 15 per cent of the cases die with excessive retentions, 70 per cent with marked retentions, while 15 per cent have normal figures in the blood. These figures are conclusive and indicate that some *variable* factor can be looked for as the cause. During the intercurrent period, 40 per cent have moderate retentions, 60

per cent are normal. Even the slight retentions during the intercurrent period probably are not of renal origin. The explanation can be sought in some physiologic disturbance in the kidney from the arterial disease or from some extrarenal cause, as cardiac decompensation from the same pathology. Nor does this factor act with any uniformity. In one instance, thirty days before death there is a note of a marked retention, though only ten days before the blood figures are normal. In another case, the day before death there is a moderate retention with a record of a marked retention five days before. From these nitrogen figures, it is also evident that the insult to the circulation from renal damage is less severe in the decrescent type of arteriosclerosis than in the precapillary type of the disease.

In a pathologic study of the vascular diseases of the kidney, it is essential (for reasons given in another communication[1]), to have a grouping between the precapillary type and the decrescent cases. As the name suggests, the features of this group resemble in various phases the one or the other. The same might be expected of the nitrogen metabolism. An analysis of the figures bears out this supposition. The slight structural changes seen in the kidneys of individuals suffering with this phase of arterial disease, play a negligible part in the disturbance of the nitrogen equilibrium. The terminal retentions resemble more closely those seen in the decrescent type. The higher number of normal figures during this period, however, approximates the figures seen in the pure type of precapillary sclerosis. The intercurrent retentions present the same variability as noted in the decrescent type. The recorded figures show less normal findings, explained, probably, by the greater severity of the arterial disease.

STRUCTURELESS KIDNEY IN INTERSTITIAL NEPHROSIS

The purest type of structureless kidney is found in the interstitial nephrotic kidney with amyloid involvement of the glomeruli where they have undergone universal and complete destruction. Such cases give excessive retentions, both in the intercurrent and terminal periods. When amyloid degeneration has attacked the glomeruli and some of the histologic features are still preserved, however, though the condition is diffuse, the nitrogen function is still maintained and these individuals die with greatly damaged kidneys, presenting during life *normal* blood findings (Case X-2 of series).*

From the foregoing, it is evident that the structureless kidney resulting either from a chronic glomerulitis, a precapillary sclerosis or an interstitial nephrosis (complicated with diffuse destruction of the glomeruli) is *always attended* with gross nitrogen disturbance in the blood. The converse of this, of course, is not true, namely, that high nitrogen retentions do not necessarily imply a structureless kidney.

It is apparent that vascular disease of the kidney is not primarily the cause of nitrogen retentions in the blood. A study of the nitrogen findings in primary tubular disease of the kidney demonstrates conclusively that the tubules play an unimportant part. In the acute degenerative types, the bloods

*A similar case is reported by Floyd in an article in the March number of the "American Journal of the Medical Sciences" of the current year.

were normal, in a few cases coming to autopsy. In the interstitial types, which represent as complete destruction of the tubules as seen anywhere in kidney pathology, and in which the glomeruli remain practically unattacked, all the blood records during the intercurrent period are normal. During the terminal period, there are records of moderate retentions in two cases, one four days before death, the other only one day. Again we note the influence of some extrarenal factor during this period but with much less effect, however, in this type of kidney lesion. Only in the primary interstitial types do the above figures hold true. The most common type of interstitial nephrosis follows disease of the tubules, secondary to some focus of infection in the body or some toxic agent produced by some metabolic disturbance. The nitrogen balance in the blood is dependent upon this extrarenal pathology. This is very well demonstrated in two cases of diabetes, in elderly individuals. One, suffering with gangrene of the extremities, had a moderate retention in the blood during the intercurrent period, dependent upon the decrescent arteriosclerosis with which she was suffering. The other had an excessive terminal retention, commonly seen in cases of decrescent arteriosclerosis.

It would appear that diffuse disease of the glomeruli is a *direct* renal cause for nitrogen retention in the blood, were it not for the outstanding example of the nephrotic interstitial kidney with partial amyloid involvement of the glomeruli and no nitrogen disturbance (Case X-2 of series). It seems, however, safe to state that *diffuse destruction* of the glomeruli is a *direct renal* cause for nitrogen retention. The same type of interstitial nephrotic kidney in which the glomeruli are totally destroyed with amyloid complication and not a vestige of normal histologic features is to be seen, is attended with high nitrogen figures in the blood (Case No. 574 of series).

It is interesting to speculate why *diffuse disease* of the glomeruli, as exemplified in the structureless kidneys of a glomerulitis and of a precapillary sclerosis, is *always accompanied* with a high nitrogen in the blood. In the structureless glomerular kidney, many of the glomeruli are totally destroyed, practically all are altered and a small proportion remain fairly intact; a pathology which technically could carry on the nitrogen metabolism with a very low reserve. But associated with the renal pathology is the cardiac hypertrophy with varying degrees of interstitial changes. Renal pathology is always a load for the circulation and a point is reached where the heart can no longer stand the added insult of the kidney, resulting in the circulatory break. This indirect factor overcomes the low functioning power of the few intact glomeruli and the nitrogen equilibrium is disturbed. Undoubtedly there are instances where the circulation is unyielding and the nitrogen metabolism remains unaffected until all the glomeruli are destroyed.

Diffuse exudative inflammation of the glomeruli is associated often with a nitrogen disturbance. The sudden strain on the circulation, the oliguria, the mechanical interference with the urinary outflow are the factors, along with the low functioning powers of the glomeruli, which induce the nitrogen decompensation. If the pathology goes on to repair (as often is the case), no permanent disturbance remains. If productive inflammation ensues, how-

ever, the functioning powers of the glomeruli are permanently reduced and unless well compensated for by a hypertension and good cardiac hypertrophy, a retention results; this in turn becomes an added load and, with the progressive renal pathology the circulation soon gives way.

In the structureless kidney of a precapillary sclerosis, most of the glomeruli are altered, many totally destroyed, but a large proportion remain intact. When it is remembered, however, that this is primarily an arterial disease, usually of an intense degree, and that the circulation is embarrassed to a point that any added burden, as renal damage, can easily induce a gross decompensation, it is not surprising that even slight structural changes in this type of kidney cause a break in the nitrogen metabolism. For this reason, the glomeruli in this type are seldom seen with as widespread destruction as in the glomerular kidney. It perhaps is true that enough glomeruli remain intact even in the type designated as "structureless" to carry on the nitrogen metabolism, but the vascular pathology (by far the severest noted in arterial disease) is a decompensating factor which overcomes any functioning power of the kidney which may remain. In this sense, this type of kidney is not a direct renal cause in the nitrogen derangements.

KIDNEY PATHOLOGY DIRECTLY INFLUENCES THE NITROGEN BALANCE BY DIFFUSE DESTRUCTION OF THE GLOMERULUS

This condition is fulfilled by the interstitial nephrotic kidney with complete amyloid involvement of the glomeruli. In glomerulitis, complete diffuse destruction is seen with a complete break in the nitrogen metabolism, but generally, it is the associated pathology—cardiac hypertrophy (a direct result of the primary disease of the kidney)—which initiates the nitrogen break and death, before all the glomeruli succumb. In this sense, the structureless kidney of glomerulitis is a direct renal cause for the nitrogen disturbance.

The interstitial nephrotic kidney, not complicated with amyloid degeneration, is not directly responsible for any nitrogen disturbance. No known associated pathology is demonstrable which can account for the slight terminal retention noted occasionally in individuals suffering with this kidney lesion. The explanation may be found in a derangement of the water metabolism which is a common accompaniment.

The preponderance of evidence in experimental physiology teaches that one of the functions of the tubules is active secretion of the waste nitrogen. In accordance with this theory, nitrogen retention in the blood should occur with disease of the tubules, and it has been recently asserted[3] that such retentions warrant a diagnosis of tubular involvement of the kidney. The most striking pathologic example is found in bichloride poisoning where the kidneys present tubular necrosis with the other radicles of the kidney surprisingly normal, the patients dying with high nitrogen figures in the blood. In the early studies, it also occurred to us that the theory built upon experimental data could be corroborated by correlating the nitrogen and pathologic findings. This was further borne out by the belief that in the structureless glomerular kidney (invariably dying with high blood nitrogens and always accompanied with tubular destruction) the cause of the reten-

tions lies in disease of the tubules. It was not until we studied more intensively the nephrotic kidney, especially the interstitial type, that the fallacy of this conception became apparent.

The question then arises, why do we get excessive retentions with the bichloride kidney? It has been our experience that a complete anuria is present during the first few days, following a poisonous dose of bichloride. For this reason, it was surprising to note in a recent article[3] that the urinary output is normal. It has been our impression that the nitrogen retention was due to the anuria. This observation is substantiated by the figures given by Gorke and Toeppich[4] in a report of a case which lived over a month. There was a complete anuria until the fourth day. On the fifth day, the nonproteid nitrogen was 158 mg. per 100 c.c. of blood. The patient then began voiding increasing amounts of urine and by the twenty-third day, there was a definite polyuria. On the thirty-fifth day, the nonproteid nitrogen was 42 mg., the next day 39 mg. The patient died on the forty-fifth day. The anuria, according to the authors, is due to the mechanical plugging of the lumen of the tubules with the dedritus from the necrosed tissue.

SUMMARY

Though technically diffuse *destruction* of the glomeruli is the only renal cause for a nitrogen retention in the blood, it has been shown that the structureless kidney of a chronic glomerulitis—characterized by diffuse *disease* of the glomeruli—should be so regarded.

All kidney pathology dependent upon a primary arterial disease influences the nitrogen metabolism in an indirect way. It is the added burden of the kidney pathology which makes the kidney a factor. In the decrescent and intermediary types, the insult to the circulation is a very variable one. In the precapillary type, kidney pathology uniformly causes a nitrogen disturbance and the so-called structureless type is always accompanied with high retentions.

Neither structural changes in the kidney independent of their origin, nor diffuse disease of the tubules, can be regarded as a direct source of a nitrogen disturbance.

Amyloid disease of the glomeruli only when completely destroying the tuft induces a nitrogen retention.

It is hoped that the conception of renal pathology and its relation to nitrogen disturbance in the body as outlined above, will stimulate a renewed interest in the diagnosis of kidney disease based upon organic pathology.

REFERENCES

[1]The material upon which this paper is based has been collected by the late Dr. John R. Larkin and myself over a period of eight years. A monograph is in preparation in which a complete discussion of the kidney pathology is undertaken with the view of correlating the clininal and metabolic phases of the disease.

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THE CAPILLARY BLOOD PRESSURE IN ARTERIAL HYPERTENSION AND IN NEPHRITIS*

By Ernst P. Boas, M.D. and Isidor Mufson, M.D., New York

LAST year one of us reported studies of the capillary blood pressure in 23 cases of arterial hypertension.[1] It was shown that patients with hypertension fall within two groups that cannot be differentiated clinically; those with high, and those with normal or low capillary pressures. Further analysis did not reveal any explanation of this phenomenon.

It was thought advisable to repeat the original observations on the same patients at the end of a year. In this paper we wish to report the results of these studies. In addition to the 23 cases first reported, 22 other patients with hypertension have been examined. We were able to reexamine 19 of the 45 cases after an interval of from 6 to 15 months. Of the remaining 26 cases 13 have died, 8 of whom have come to autopsy. Reports concerning the 13 others were received through the social service department of the hospital, following a personal interview with the patient.

Tables I and II present the facts that were ascertained.

Table III is a summary of the preceding tables.

It is worthy of note that the capillary readings on the individual cases taken many months apart correspond quite closely. In the few cases in which there has been a significant change in the capillary pressure a definite explanation for the new readings is apparent, as will be discussed below. The constancy of the readings speaks well for the accuracy of the method employed, and gives evidence that the persistent high capillary pressure is due to some significant physiologic or pathologic reaction of the body.

Of the total of 45 cases, 17 had high capillary blood pressures. The mortality of the patients with high capillary pressures was much greater than of those with low readings; 8 out of 17 of the former, and 5 out of 28 of the latter having died.

It seems that those patients with a low capillary pressure had been ill for a longer time than those with high pressures, but too much emphasis cannot be placed on this observation because of the great difficulty in ascertaining the date of onset of the illness.

An average of the figures indicating the number of months that patients have survived since their first examination would be meaningless, because of the fact that they were not examined at the same time—some were first seen 17 months ago and some 5 months ago. The patients both with high and with low capillary pressures who died showed renal impairment more frequently than did those who survived.

*From the Medical Division of the Montefiore Hospital for Chronic Diseases.
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TABLE I

PATIENTS WHO HAVE DIED

HIGH CAPILLARY PRESSURE	DURATION OF ILLNESS (YRS.)	DURATION OF LIFE AFTER 1ST CAPILLARY PRESSURE (MONTHS)	KIDNEY FUNCTION	B. P. SYSTOLIC	B. P. DIASTOLIC	CAPILLARY PRESSURE	CAUSE OF DEATH
Case 2	8.00	17	Good	235	120	27 – 48	Cerebral hemorrhage
Case 3	1.25	11	Fair	244	150	35 – 85	Cardiac insufficiency
Case 5	4.50	9	Good	212	110	31 – 54	Pyelonephritis (autopsy)
Case 38	1.50	2	Poor	260	130	26 – 54	Uremia (Autopsy)
Case 39	1.50	1	Poor	210	160	25 – 85	Uremia
Case 67	3.00	2	Fair	170	110	50 – 73	Thrombosis, sepsis (autopsy)
Case 77	2.00	5	Poor	240	130	20 – 70	Pulmonary edema, Uremia (?)
Case 81	10.00	0.3	Poor	210	140	42 – 55	Uremia (Autopsy)
Average	3.97	5.9		223	131		
LOW CAPILLARY PRESSURE							
Case 11	3.66	2	Fair	180	95	7 – 17	Bronchopneumonia (autopsy)
Case 22	4.00	8	Fair	210	120	4 – 26	Cerebral Hemorrhage (autopsy)
Case 41	15.00	5	Fair	235	120	25 – 31	Cardiac Failure, Coronary Thrombosis (autopsy)
Case 13	4.00	8	Fair	210	85	10 – 35	Cardiac Failure, Asthenia
Case 19	5.00	1.5	Poor	195	104	5 – 10	Uremia (Autopsy)
Average	6.3	4.9		206	105		

Four patients with high and four patients with low capillary pressure came to autopsy. No characteristic features were noted in either group of cases. They all exhibited granular kidneys of varying degrees. The amount of kidney destruction was no greater in one group than in the other, nor was there any difference in the nature of the lesions or in the relative involvement of glomeruli and tubules. In one case (67) with high pressure, the kidney, as a whole, and particularly the glomeruli, were in excellent condition. The appearance of the small blood vessels was studied, not alone in the kidney but in the other organs as well, particularly in the spleen and pancreas, as suggested by Evans.[2] In some cases the intimal thickening of the arterioles was extreme, in others moderate, but all degrees of sclerosis were found in both categories of cases. Pathologic anatomy, therefore, does not support Kylin's theory that a high capillary pressure is indicative of glomerulonephritis.

A comparison of the causes of death of patients with high and with low capillary pressure offers no further information. It is true that of the 8 high pressure cases, 4 died of uremia, but one patient with low pressure

TABLE II

LIVING PATIENTS

HIGH CAPILLARY PRESSURE	DURATION OF ILLNESS (YEARS)	INTERVAL BETWEEN 1ST & 2ND OBSERVATIONS (MONTHS)	KIDNEY FUNCTION 1ST	KIDNEY FUNCTION 2ND	BLOOD PRESSURE SYSTOLIC 1ST	BLOOD PRESSURE DIASTOLIC 1ST	BLOOD PRESSURE SYSTOLIC 2ND	BLOOD PRESSURE DIASTOLIC 2ND	CAPILLARY PRESSURE 1ST	CAPILLARY PRESSURE 2ND	CONDITION OF PATIENT
Case 6	2.5	12	Good	Good	220	110	220	120	48–70	28–65	Same
Case 4	1.0	14	Fair	Fair	216	120	220	140	20–90	20–60	Same
Case 1	6.0	14	Good	Good	130	90	135	85	21–41	35–37	Same
Case 17	6.0	14	Fair		196	120	228	114	26–65		Feels better
Case 21	3.0	15	Fair		164	90	135	80	21–49	8–22	Same
Case 70	5.0	5	Good	Good	210	124	250	130	28–54	30–55	Same
Case 71	3.0	7	Good		210	120			30–53		Good
Case 73	2.0	6	Good		175	95			55–83		Better
Case 16	1.5	6	Good		216	120	234	134	18–60		Same
Average	3.3				193	109.9	203	115			
LOW CAPILLARY PRESSURE											
Case 7	12	12	Good	Good	174	110	170	92	12–27	15–20	Same
Case 8	2	15.0	Good		164	94	160	100	4–10	5– 8	Cardiac failure
Case 8-A	1	12	Fair	Fair	180	60	150	50	20–27	20–25	Same
Case 9	8	13	Good	Good	145	90	170	90	2– 6	10–12	Same
Case 10	3	16	Fair		175	85			5– 7		Same
Case 10-A	11	12	Good		145	75	170	95	18–33	14–55	Same
Case 12	0.5	16	Fair		180	100			10–20		Same
Case 14	4.0	13	Fair		204	140	230	130	6–36	20–40	Same
Case 15	3.0	13	Good		208	100	216	94	7–13		Same
Case 18	Many years	14	Good		174	72			4–11		Worse
Case 20	1	15	Good		226	100	235	120	12–38	39–58	Same
Case 23	4	13	Good		238	138			16–24		Same
Case 37	2	7.5			150	100	180	90	10–17	18–25	Same
Case 40	3	11	Good		203	140	220	140	14–23	8–21	Same
Case 42	10	1.5			180	90	130	105	7–26		Same, Dyspnea Edema of Legs
Case 43	1	7	Good	Good	190	100	200	90	5–27	12–22	Same
Case 44	1	7	Good	Good	160	110	120	90	5–10	5–15	Same
Case 45	18	7	Good		120	85	120	90	18–20	10–20	Same
Case 46	2.5	8	Good		220	112	200	110	15–25*	37–70	Same
Case 64	2.5	7	Good		195	115			10–30		Poor—bedridden incontinent
Case 68	5	5.5	Poor	Poor	170	105	214	130	22–30	15–50	Same
Case 74	0.5	7			170	112	190	100	3–25	5–30	Same
Case 47	1.5	8			220	130			17–23		Same
Average	4.4				182	103	179	99			

*At the first examination two readings of 55 were obtained. The other 9 readings were below 25.

TABLE III

HIGH CAPILLARY PRESSURE	NO.	AVERAGE DURATION OF ILLNESS (YEARS)	DURATION OF LIFE SINCE 1ST CAPILLARY PRESSURE (MONTHS)	KIDNEY FUNCTION GOOD	KIDNEY FUNCTION IMPAIRED	AVERAGE BLOOD PRESSURE 1ST SYSTOLIC	1ST DIASTOLIC	2ND SYSTOLIC	2ND DIASTOLIC
Alive	9	3.3		6	3	193	110	203	115
Dead	8	3.97	5.9	2	6	223	131		
LOW CAPILLARY PRESSURE									
Alive	23	4.4		14	5	182	103	179	99
Dead	5	6.3	4.9	0	5	206	105		

died of the same cause. The cases are too few to warrant any deductions, since they show so great a variability.

The most significant relationship established by our observations appears to be that between the capillary pressure and the diastolic blood pressure. On the average the diastolic pressure is higher in those patients with high capillary pressures, particularly in those who have succumbed to their disease since they were first studied. With 2 exceptions, and in these the capillary pressure was not very high, the lowest diastolic pressure was 110 mm. Hg. However, many patients with a diastolic pressure over 110 exhibited a normal capillary pressure (see cases 7, 14, 22, 23, 40, 41, 44, 46, 47, 64).

Three patients (cases 10-a, 20, 68), in whom the capillary pressure was normal last year, showed a significant rise in capillary pressure on reexamination from 8 to 15 months later. All three patients showed a concomitant rise in the blood pressure—particularly in the diastolic pressure. Clinically there was little change in their condition. One patient (case 21) exhibited a drop in capillary pressure with a corresponding drop in blood pressure. In one individual (case 46) there was a rise in the capillary pressure with no change in the blood pressure.

In order to obtain further evidence of the relationship of the diastolic pressure to the capillary pressure, we studied a series of patients before and after the administration of nitroglycerine. The following are protocols of typical experiments:

PATIENTS WITH HIGH CAPILLARY PRESSURE AND HYPERTENSION

	CAPILLARY PRESSURE	B. P.
CASE 4.		
Preliminary readings:	50, 45, 45, 40, 40, 55, 45	210/140
Nitroglycerine gr. 1/50 by hypo:		
After ½ minute	50, 45	
" 1 "	22	
" 2 "	10, 8	
" 7 "	26, 35, 24, 40	
" 9 "	35, 30, 20, 33	
" 12 "	35, 40	190/130
CASE 6.		
Preliminary readings:	35, 40, 38, 30, 38, 50	210/140
Nitroglycerine gr. 1/50 by hypo:		
After ½ minute	30, 20	
" 1 "	22	
" 2 "	22, 20	
" 5 "	30, 35, 40	
" 8 "		210/140

Average 4.4 | 182 | 103 | 179 | 99 |

*At the first examination two readings of 55 were obtained. The other 9 readings were below 25.

PATIENT WITH NORMAL CAPILLARY PRESSURE AND HYPERTENSION

Case 94.	Capillary Pressure	B. P.
Preliminary readings:	4, 15, 12, 18, 25, 29, 20, 20, 28	240/100
Nitroglycerine gr. 1/50 under tongue		
After 11 minutes	14	
" 15 "		
" 22 "	12	230/100
" 30 "	10	230/95
" 30 "	10	
" 42 "	16	200/100
" 50 "	15	

The test was performed on 8 patients. The blood pressure was taken on one arm and the capillary pressure on the other, in order to avoid any possible artificial circulatory disturbance. However, in a number of cases it was noted that the inflation of the blood pressure cuff on one arm was followed by an arrested flow, and by the disappearance of a number of capillaries of the finger on the opposite side. This must be a reflex action and analagous to the change in blood flow in one hand when the other is subjected to varying temperatures.[3] In order to avoid this factor, blood pressure readings in most instances were taken only at the beginning and at the end of the experiment. Following the administration of nitroglycerine no dilatation of the capillaries of the finger was noted, but in a number of cases there was a definite acceleration of the blood flow. In other instances no change in the rapidity of the blood stream was apparent, but in no case was it retarded.

The mechanism underlying the relationship between the capillary pressure and the blood pressure remains obscure. It is difficult to understand how a high blood pressure, due presumably to a constriction of the arterioles, can cause a high capillary pressure. As a matter of fact, arteriolar contraction results in a low pressure in the capillaries peripheral to the constriction. Of course, we are measuring the capillary pressure only in very restricted portion of the total capillary bed, and it is conceivable that a constriction or dilatation of the splanchnic vessels may be accompanied by a dilatation or constriction respectively of the arterioles of the extremities.

The results that we obtained following the administration of nitroglycerine do not support this view. Although Cushny[4] states that the nitrites act more on the vessels of the abdominal organs and of the head, than on those of the extremities, and that in the skin only the vessels of the head and neck are dilated by the drug, Hewlett[5] has shown by means of a plethysmograph that there is an increase in the volume of the arm, and Stewart[6] has demonstrated an increased blood flow through the hand following the administration of nitroglycerine. Finally, Laubry and Meyer[7] and Carrier[8] observed microscopically dilatation of the capillaries of the fingers after the administration of nitrites. Carrier also described a very rapid blood flow in the capillaries after inhalation of amyl nitrite. Moog and Ambrosius[9] observed no capillary reaction in normals following the administration of nitroglycerine; but in patients with hypertension they noted within five to seven minutes after the administration of nitroglycerine a drop in blood pressure,

and a rapid continuous streaming of the blood in the capillaries lasting one to two and one-half hours.

In our experiments we observed a great increase in the velocity of the blood flow in the capillaries, as well as a fall in the capillary blood pressure, following the subcutaneous injection of nitroglycerine. The increase in the blood flow indicates a dilatation of the arterioles. Such a dilatation is usually accompanied by a rise in capillary pressure,[10] as is observed, for instance, after warming the hand. In spite of the fact that nitroglycerine markedly lowers the blood pressure, the head of pressure in the arteries is still sufficient in comparison with the capillary pressure to be transmitted through the dilated arterioles to the capillaries, and prevent a lowering of the capillary pressure. The parallel fall of brachial and capillary blood pressures may be explained by an effect of the drug on the arterioles and capillaries simultaneously. A dilatation of the patent capillaries with the opening of many capillaries that have been temporarily closed would accommodate the increased blood flow at a lower pressure. It is evident, therefore, that the capillary blood pressure in these patients is not dependent on the diastolic blood pressure.

It is impossible, at present, to ascertain to what degree the fall in capillary pressure determines the fall in diastolic pressure. That it may have this effect in extreme cases we know from the pathologic physiology of shock, in which the fall in blood pressure and the failing circulation have been proved to be due to a paralytic dilatation of the capillaries. There is no unassailable evidence that the converse of this holds true, that a constriction of the capillaries may cause an elevation of blood pressure—but it certainly must be envisaged as a distinct possibility. One interpretation, therefore, might give to the capillaries the controlling influence and assume that the high capillary pressure is primary, and determines the level of the diastolic pressure. Such an elevation of the capillary pressure might be conditioned by a narrowing of the capillaries, or by a constriction of the venules, or of the venous portion of the capillary loop. It has been shown that the capillaries of the skin of normal individuals can exert an active constrictive force of at least 30 mm. Hg.[11] When the vast size of the capillary blood bed is taken into consideration, it is easy to visualize the effect that a general capillary constriction might have on the blood pressure. Finally, the high arterial and capillary pressures may be independent of one another, but determined by a common factor.

We have had the opportunity of studying four patients with acute or subacute nephritis. Two of these had scarlatinal nephritis and were seen through the courtesy of Dr. E. Giddings at the Willard Parker Hospital. Both patients were in the third week of their nephritis (Table IV).

These readings were taken with the patient lying in bed with the hand at heart level. Danzer and Hooker[12] have shown that the capillary pressure determinations vary with the posture of the patient during the examination, and that the pressure in the recumbent posture is from 3 to 5 mm. Hg. lower than when the patient is sitting. Allowing for this correction the

TABLE IV

DATE	CAPILLARY PRESSURE	BLOOD PRESSURE	CAPILLARY MORPHOLOGY	REMARKS
	WM. B., MALE, AGE 10, SCARLATINAL NEPHRITIS			
11/16/22	21, 26			
	18, 25	120/88	Capillaries few in number, small, many convoluted with figures of 8.	General edema. Urea N. 38 Uric acid 5.6
	18, 25			
11/24/22	27, 18			
	22, 20	140/100	Same	The same.
	21, 25			
12/ 4/22	20, 16			
	18, 26	120/70	Capillaries more numerous and more convoluted.	Edema has largely disappeared.
	30, 23			
	23, 25			
	25, 25			
	R. W., FEMALE, AGE 8, SCARLATINAL NEPHRITIS			
12/ 4/22	28, 32			
	23, 40	145/100	Capillaries long, not convoluted.	Moderate edema.
	21, 35			
	24, 12			
	26, 25			

readings which we obtained are at about the upper limit of normal, but do not reach the high figures, 35 to 40 mm. Hg., reported by Kylin.

The third case (hospital number 9372), which we saw through the courtesy of Dr. A. I. Ringer, was one of subacute nephritis of three months' standing which followed a sore throat. The patient, a young girl of nineteen, was very ill with a marked anemia, urine of low specific gravity, showing much albumin and many casts, blindness from detachment of the retinas, bleeding from the mucous membranes, hypertension and marked nitrogen retention in the blood. The capillary pressure readings were 14, 18, 30, 19, 17, 5, 15. The blood pressure was 185/85. The capillaries were numerous and irregularly convoluted. This patient, too, was examined in the recumbent posture.

TABLE V

DATE	CAPILLARY PRESSURE	BLOOD PRESSURE	CAPILLARY MORPHOLOGY
12/ 1/22	35, 25		
	42, 38	110/80	Simple long loops.
	32, 35		No convolution.
	25, 42		
	26		
12/ 8/22	22, 31, 32		
	30, 35, 31		
	25, 38, 25	110/90	Same
	32, 25, 32		
	15, 16, 32		
1/ 4/23	30, 32, 27		
	30, 38, 26	120/90	Same
	35, 37, 31		
1/ 8/23	41, 43, 34		
	41, 35, 34		
	41, 30, 34	125/95	Same
	41, 30, 33		
	39, 23, 29		
	36, 23, 25		
	43, 23, 31		
	43, 34, 29		
3/ 1/23	42, 38, 38		
	35, 42, 33	105/70	Same
	38, 38, 38		

On the day on which she was examined the blood urea N was 180 mg. per 100 c.c. She died two weeks later with a final blood urea N of 227 mg.

The fourth patient, a girl fifteen years of age, had been ill for about 2 years. On examination she showed a fair nutrition, no anemia, normal blood pressure, no cardiac hypertrophy, much albumin with casts and red blood cells in the urine, and moderately impaired kidney function without nitrogen retention. Observations on the capillary pressure made with the patient in a sitting posture are given in Table V.

This last patient is of particular interest because she is the only one, among over 100 patients of all sorts whom we have examined, who presents a high capillary pressure with an essentially normal blood pressure. A study of more patients with acute and subacute nephritis is desirable.

SUMMARY

Forty-five patients with arterial hypertension were studied over a period of from 6 to 15 months. Of the 17 with high capillary pressures 8 have died; of the 28 with low capillary pressures only 5 have died.

Necropsy studies and observations on 2 patients with scarlatinal nephritis do not support the theory that high capillary pressures are indicative of glomerulonephritis.

In most patients the capillary readings taken at long intervals are constant. With few exceptions, high capillary pressures are accompanied by high diastolic pressures, and in these cases there is a certain parallelism between the two pressures.

In patients with hypertension nitroglycerine lowers both the arterial and the capillary blood pressures.

A case of subacute nephritis with normal blood pressure and high capillary pressure is reported.

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CHANGES IN THE BLOOD OXYGEN FOLLOWING THERAPEUTIC BLEEDING IN CARDIAC PATIENTS*

By Samuel B. Grant, M.D., Boston, Mass.

BLEEDING has been practiced for hundreds of years as a method of treating acutely decompensated cardiac patients, and its value has been well established by abundant clinical observations. However, there has been very little data reported concerning the changes this treatment produces in the disturbed circulation, and it seems worth while to record a few observations on the oxygen content of the arterial and venous blood before and after therapeutic venipuncture.

The group studied consisted of seven acutely decompensated cardiac patients, who were to be bled by the house physicians purely as a therapeutic measure. Samples of blood were taken under oil and without stasis from the anticubital vein (Lundsgaard[1]) and from the radial artery (Stadie[2]) immediately before the venipuncture. In some instances samples were taken three to five hours before, as well as immediately before, bleeding, in order to establish a better base-line with which to compare the subsequent changes. In from one and one-half to three and one-half hours afterwards samples were taken again, using the same artery and vein. There was naturally some difficulty in getting cooperation with this type of patient, and at times the only available vein was rendered useless by the bleeding (although this was always done by inserting a large needle into the vein and a skin incision was never necessary). If any considerable difficulty was experienced in obtaining the samples the attempt was discontinued, rather than subject the patient to this added discomfort. Accordingly, complete data was obtained in only five patients. As a rule it was easier to tap the radial artery than to get into the anticubital vein without a tourniquet, so that the incomplete data concerns the venous blood. The samples were immediately analyzed in duplicate for oxygen content and oxygen capacity, using the modified Van Slyke apparatus and the method of Van Slyke and Stadie.[3]

Before entering upon any discussion it would be well to present the cases in some detail.

Medical No. 19479.—A tailor, aged 59, had a history of gradually increasing dyspnea for three years, becoming quite severe, with orthopnea, just before entering the hospital.

Examination showed marked orthopnea, cyanosis, enlarged outline of cardiac dullness, regular rhythm, and signs of aortic insufficiency. Blood pressure was systolic 204, diastolic 60. Rales were present throughout the lower half of the lungs, and the liver was enlarged to 6 cm. below the costal margin.

Four hundred c.c. of blood were removed by venipuncture. Samples of arterial and venous blood were obtained immediately before and one and one-half hours afterwards.

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The arterial oxygen saturation increased from 87.1 per cent to 92.1 per cent, and the venous oxygen saturation increased from 79.0 per cent to 84.9 per cent after the bleeding. The coefficient of ultilization of oxygen, i.e., the difference between the arterial and venous oxygen saturations, decreased from 8.1 to 7.2, indicating a slightly more rapid flow of blood through the arm.

The patient improved clinically, cyanosis decreased, and the rales had practically disappeared from the lungs on the following day. One month later he was discharged fairly well compensated, able to walk about quietly without dyspnea. Distinctly against advice, he immediately began active exercise, such as chopping wood, and was forced to reenter one week later, severely decompensated again, with orthopnea, cyanosis, pulsus alternans, moderate edema of the legs, and signs of fluid in the right chest.

Fluid (1650 c.c.) was removed from the chest, and three and one-half hours later the arterial blood was 88.7 per cent saturated with oxygen, as compared with 86.7 per cent saturation immediately before thoracentesis. The venous oxygen saturation increased from 40.3 per cent to 77.6 per cent, and the coefficient of utilization decreased from 46.4 to 11.1, indicating a distinct increase in the rate of blood flow through the arm. Clinically the patient was greatly improved, much less dyspneic, and no longer cyanotic.

MEDICAL NO. 19572.—A shoemaker, aged 49, had had severe attacks of angina pectoris three years and again one year before admission, with marked dyspnea and cough for the last year. Five days before admission he had a third anginal attack, and became extremely dyspneic. Examination showed cyanosis, orthopnea, greatly enlarged outline of cardiac dullness, with regular rhythm, weak heart sounds, and no murmurs. There were rales throughout the lungs, and the liver was enlarged to 7 cm. below the costal margin.

Four hundred c.c. of blood were removed by venipuncture. The arterial blood two hours later was 78.3 per cent saturated, as compared with 70.8 per cent saturation immediately before bleeding, indicating improved efficiency of the pulmonary circulation. The first sample of venous blood was taken when the patient was quite excited, fighting for breath, and is, therefore, not quite comparable with the samples taken later, when the patient was perfectly quiet. The venous oxygen saturation decreased from 49.3 per cent to 36.5 per cent after bleeding, and the coefficient of utilization increased from 21.5 to 41.8. The peripheral circulation must have been more rapid during the preliminary excited stage, and slower when the patient had quieted down after bleeding.

The patient improved, cyanosis practically disappeared, and the rales in the lungs had almost disappeared four days later. Samples of blood taken at this time showed normal arterial oxygen saturation (96.7 per cent), normal venous oxygen saturation (74.2 per cent) and normal coefficient of utilization (22.5).

MEDICAL NO. 19663.—A Russian tailor, aged 54, had recurrent attacks of angina pectoris for three years, and for three months had had increasing dyspnea, edema of the legs, nocturia, diarrhea, and nausea and vomiting, with a feeling of constriction in the chest and pain radiating up the neck and down the arms.

Examination showed marked orthopnea, moderate cyanosis, greatly enlarged heart, with regular rhythm and systolic and diastolic murmurs loudest at the third left intercostal space. Blood pressure was systolic 220, diastolic 110. There were moist rales throughout the lungs. The liver was enlarged to 7 cm. below the costal margin and there was moderate edema of the legs.

There was no sign of improvement for three days, and dyspnea was increasing. He was then bled 350 c.c. The arterial oxygen saturation two hours afterwards was 89.6 per cent, essentially the same as before (90.9 per cent). The venous oxygen saturation increased from 68.6 per cent to 71.1 per cent, and the coefficient of utilization dropped from 22.4 to 18.5, suggesting an increase in the volume of blood flow through the arm. The pulse improved in quality, dyspnea decreased, and the blood pressure rose to systolic 295 and diastolic 140. Digitalis was then given and the edema decreased. Patient was discharged six days later against advice, improved but still in a precarious condition.

MEDICAL NO. 19645.—A schoolgirl, aged 15, had a history of tonsillitis and rheumatic fever in early childhood, with dyspnea and palpitation after the age of nine. For four weeks prior to admission she had had pain in the epigastrium, frequent vomiting, and had been confined to bed. Examination showed dyspnea, marked cyanosis, greatly enlarged and absolutely irregular heart with signs of mitral stenosis. The liver was enlarged to 6 cm. below the costal margin. The lungs were resonant and no rales were heard. There was no edema of the extremities, but the abdomen was distended, with shifting dullness in the flanks and a fluid wave.

Three hundred c.c. of blood were removed. The arterial blood was essentially normally saturated before bleeding (93.1 per cent), as was to be expected with lungs clear and resonant, and two and one-half hours afterwards showed no change (93.2 per cent saturated). The venous oxygen saturation was 54.4 per cent and the coefficient of utilization was 38.7 before bleeding. Samples of venous blood could not be obtained after bleeding. Lundsgaard[4] has demonstrated the great variability of the venous oxygen unsaturation in patients with auricular fibrillation, presumably due to the irregularity of the systolic output of the heart, so that it would have been impossible to draw any conclusions from only two consecutive samples of venous blood in a patient with auricular fibrillation.

Clinically the patient gradually improved; under digitalis the heart rate decreased from 120 to 60, cyanosis decreased, and there was a diuresis with decrease in body weight of 3.8 kilos the first three days in the hospital. Patient was discharged improved after a month's rest.

MEDICAL NO. 19877.—A negro laborer, aged 52, with a history of chancre at the age of 22, began having dyspnea one and one-half years before admission, gradually increasing with the development of cough, precordial discomfort, orthopnea for eight months and edema of the legs for one month.

Examination revealed cyanosis, orthopnea, marked edema of the legs, greatly enlarged heart with regular rhythm and no murmurs, pulsus alternans, moist rales over the lungs, liver enlarged to 6 cm. below the costal margin, and distention of the abdomen with shifting dullness in the flanks. Blood pressure was systolic 155, diastolic 100.

Under digitalis there was a marked diuresis, but cyanosis and orthopnea persisted. On the third day 300 c.c. of blood were removed. The arterial blood before was 89.3 per cent saturated with oxygen, and exactly the same two hours afterwards. The only available vein was ruined by the venipuncture. Venous oxygen saturation before bleeding was 42.5 per cent, with a coefficient of utilization of 45.9. The patient continued to improve clinically and was discharged 33 days after admission. Repeated blood Wassermann reactions were negative. This case also showed no change in the arterial blood, which before bleeding was only a little lower in oxygen saturation than normal. If the amount of bleeding had been larger, there might have been some effect.

MEDICAL NO. 19981.—A chauffeur, aged 52, had a sudden severe knife-like pain over the precordium one year before admission, leaving a dull aching sensation for several days, but was then well until three months before admission, when he had a similar attack, associated with vomiting, and followed by persistent dyspnea which soon confined him to bed. Two days before admission his legs began to swell and dyspnea increased.

Examination showed marked prostration, moderate cyanosis, slight jaundice of the skin and sclerae, heart moderately enlarged to percussion, with weak heart sounds and no murmurs or friction rub. Blood pressure was systolic 110, diastolic 78. There was pulsus alternans. Coarse rales were present throughout the lungs. The liver was enlarged to 5 cm. below the costal margin, and there was marked edema of the legs. Before bleeding the arterial blood was 89.4 per cent saturated, and the venous blood 35.9 per cent saturated with oxygen, and the coefficient of utilization was 53.5. He was then bled 700 c.c. Two hours later the arterial blood had shown a slight decrease to 86.4 per cent saturation, but the venous blood had risen to 67.7 per cent saturation,

TABLE I

PATIENT MEDICAL NO.	TIME	OXYGEN CAPACITY VOL. %	OXYGEN CONTENT ARTERIAL VOL. %	OXYGEN CONTENT VENOUS VOL. %	OXYGEN UNSATURATION ARTERIAL VOL. %	OXYGEN UNSATURATION VENOUS VOL. %	OXYGEN SATURATION ARTERIAL %	OXYGEN SATURATION VENOUS %	COEFFICIENT OF UTILIZATION
19479	Before Bleeding	17.61	15.33	13.93	2.28	3.68	87.1	79.0	8.1
	1½ hours After Bleeding	16.83	15.50	14.29	1.33	2.54	92.1	84.9	7.2
	Before thoracentesis	16.64	14.43	6.71	2.21	9.93	86.7	40.3	46.4
	3½ hours later	15.73	13.96	12.20	1.77	3.53	88.7	77.6	11.1
19572	Before bleeding	24.39	17.26	12.02	7.13	12.37	70.8	49.3	21.5
	2 hours after bleeding	22.60	17.70	8.25	4.90	14.35	78.3	36.5	41.8
	4 days later	19.84	19.18	14.73	0.66	5.11	96.7	74.2	22.5
19663	2 days before	21.08	19.35		1.73		91.8		
	Immed. before	21.96	19.98	15.06	1.98	6.90	90.9	68.6	22.38
	2 hours after bleeding	20.50	18.36	14.58	2.14	5.92	89.6	71.1	18.46
19645	Before	16.60	15.46	9.03	1.14	7.57	93.1	54.4	38.7
	2½ hours after bleeding	15.37	14.32		1.05		93.2		
19877	5 hours before	18.74	16.57	7.94	2.17	10.79	88.4	42.5	45.9
	Immed. before	18.04	16.11		1.93		89.3		
	2 hours after bleeding	18.04	16.11		1.93		89.3		
19981	1 hour before	14.64	13.09	5.25	1.55	9.39	89.4	35.9	53.5
	2 hours after bleeding	13.56	11.72	9.18	1.84	4.38	86.4	67.7	18.7
19927	3 hours before	17.61	14.60	10.88	3.01	6.73	82.9	61.8	21.1
	1 hour before	17.19	12.77		4.42		74.3		
	2 hours after bleeding	15.56	10.21	7.10	5.35	8.46	65.6	45.6	20.0

and the coefficient of utilization had dropped to 18.7, suggesting an increase in the rate of flow through the arm.

There was immediate symptomatic relief, and cyanosis was less marked than before. On the following day, however, he began to become worse again, digitalis had no effect, and the pulse became weaker. Eleven days later, as he was gradually failing, a thrombophlebitis of the left iliac vein developed, and nineteen days after admission the patient died. At autopsy there was found infarction of the heart, chronic fibrous pericarditis, extensive infarction of both lungs, left iliac thrombosis, passive congestion of the viscera, and bilateral pneumonia.

MEDICAL No. 19927.—A mulatto, aged 68, with a history of chancre, began having dyspnea, nocturia, and slight edema of the feet seven years before admission, and was diagnosed at that time as chronic nephritis with hypertension and chronic myocarditis. Nine months before admission he became very short of breath, developing orthopnea and marked edema of the legs.

Examination showed marked dyspnea, greatly enlarged heart with weak heart sounds, normal rhythm, and slight systolic murmur at the apex. Blood pressure was systolic 176, diastolic 90. Moist rales were present over the lower half of the lungs. The liver was palpable 14 cm. below the costal margin, and there was marked edema of the legs.

Samples of blood taken three and one-half hours before bleeding showed arterial oxygen saturation 82.9 per cent, venous oxygen saturation 61.8 per cent, and coefficient of utilization of 21.1. Two and one-third hours later the arterial oxygen saturation had decreased to 74.3 per cent. He was then bled 750 c.c., and samples taken again two hours afterwards. The arterial saturation had continued to fall, reaching 65.6 per cent, and the venous blood 45.6 per cent saturation. The coefficient of utilization was 20.0. This slight decrease in the coefficient of utilization, with a change in the actual amount of oxygen utilized of from 3.72 vol. per cent before bleeding to 3.11 vol. per cent afterwards, suggests an increase in the rate of blood flow through the arm. However, the patient rapidly failed, and died thirty-six hours after the venipuncture. At autopsy there was found marked edema and congestion of the lungs, markedly hypertrophied and dilated heart, with scarring of the myocardium and normal heart valves, moderately sclerosed but patent coronary arteries, passive congestion of the liver, and chronic nephritis.

DISCUSSION

It has been very well shown by Gesell[5] in experiments on normal dogs that the volume-flow of blood through the salivary gland and through striated muscle is considerably decreased following hemorrhage. Meek and Eyster[6] studied the effect of hemorrhage on the diastolic size of the heart as shown by x-ray, using normal dogs as subjects, and found no appreciable change until 2 per cent of the body weight in blood had been lost. Arterial and venous blood pressures and the heart rate also remained unchanged. Under artificial respiration with the chest open, the cardiometer showed no change in the volume output per minute or per beat, until about 1 per cent of the body weight of blood had been lost, after which there was a rather abrupt decrease.

In decompensated cardiac patients, however, the circulation is in an entirely different condition from that in the normal animal. The outstanding feature in such a decompensated circulation is stasis, with engorgement of the venous channels throughout the body, and slow rate of flow through the capillaries. Each unit of blood must give up a larger proportion of its oxygen than normally, in order to meet the oxygen consumption of the tissues, and this is one of the primary factors in the production of cyanosis (Lundsgaard and Van Slyke[7]). A reduction of the blood volume by bleeding would tend to relieve this passive congestion of the tissues, and facilitate a more rapid flow of the blood through the capillaries. Theoretically then, each unit of blood passing through the capillaries would no longer be as depleted of oxygen as before, and cyanosis due to this cause should decrease. This is what was actually found in the cases presented above; each unit of blood was giving up less oxygen after bleeding than before, i.e., the coeffi-

cient of utilization of oxygen decreased. Two cases showed a definite increase in the percentage of oxygen saturation in the arterial blood. This was presumably due to a mechanism similar to that producing the change in the coefficient of utilizaton, i.e., a decrease in the pulmonary engorgement with consequent better aeration of the lungs and, with the more rapid circulation, possibly a decrease in the pulmonary edema. Peabody, Blumgart and Drinker[8] have shown that pulmonary congestion reduces the vital capacity of the lungs. Three cases had only a slightly lower arterial oxygen saturation than normal, and showed no change in this respect after bleeding. One case had a rapid fall of arterial oxygen saturation before bleeding, which continued after bleeding, with death a short time later. Apparently the heart was failing too rapidly to respond to any treatment. Another case had a very slight decrease of the arterial oxygen saturation after bleeding, while the venous oxygen saturation was improving. This patient also died later. One of the patients who showed improvement after venipuncture reentered the hospital later with fluid in the right chest. Thoracentesis, with removal of 1650 c.c. of fluid, was followed by an increase in the oxygen saturation of both the arterial and venous blood, due undoubtedly to improved aeration of the lungs and increased efficiency of the heart. The presence of a large amount of fluid in the pleural cavity might mechanically retard the return of venous blood to the right heart, thus tending to increase the venous oxygen unsaturation.

SUMMARY

1. Therapeutic bleeding of decompensated cardiac patients produced an improvement in the peripheral circulation, as evidenced by a decrease in the coefficient of utilization of oxygen.

2. When the arterial oxygen saturation was considerably below normal, and the heart not so seriously damaged as to be unable to respond to treatment, bleeding was followed by an increase in the arterial oxygen saturation, indicating improved efficiency of the pulmonary circulation.

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COMPARATIVE ANTIPHLOGISTIC EFFECTS OF SALICYLATE, CINCHOPHEN AND NEOCINCHOPHEN IN EXPERIMENTAL EDEMA OF THE HEAD AND NECK*

By P. J. Hanzlik, M.D., and M. L. Tainter, A.B., San Francisco, Calif.

AMONG other beneficial effects produced by salicylates, cinchophen and neocinchophen in the treatment of acute rheumatic fever is the prompt amelioration of the objective signs of inflammation, including the swelling and edema of the joints, the so-called antiphlogistic action of these drugs. This relief might occur from a direct action upon the inflammatory process (organotropic action), or indirectly by removal of the etiologic factor (etiotropic action), i.e., through antiseptic or bactericidal actions. Since the etiology of rheumatic fever is unknown, the etiotropic action of these agents cannot be tested satisfactorily. Therefore, this leaves a test of the organotropic action of these agents for consideration.

Some idea of the organotropic action should be obtainable from the response of experimental inflammatory lesions to these drugs. An organotropic action by cinchophen and salicylate is suggested from their power to alter (increase) renal permeability, either vascular or cellular, toward certain metabolites (uric acid) demonstrated by Folin and Lyman[1] for cinchophen, by Fine and Chace[2] for salicylate, and by Myers, Killian and Simpson[3] for cinchophen and neocinchophen. A beneficial action for cinchophen has been strongly claimed by Wiechowski and Starkenstein[4] in at least two experimental inflammatory edemas, namely, in the croton oil inflammation of the skin in cats, and in mustard oil chemosis in rabbits. The views of Wiechowski and Starkenstein, and of Starkenstein[5] have been the basis for claims of extraordinary antiphlogistic effects in a variety of clinical conditions, and for commercial exploitation by interested manufacturers. Opposing evidences have been brought forward by Heubner and Gildemeister[6], who could not confirm the alleged antichemotic action of cinchophen in cats, and by Lacquer and Magnus[7], who found cincophen and salicylate not to be beneficial in the pulmonary edema produced by phosgene in cats. In fact, the latter investigators found that cinchophen was detrimental to, that is, it increased the mortality of, phosgenized cats.

In view of the conflicting claims and evidences, it was thought desirable to seek further evidence of the actions of these agents in another inflammatory edema produced systemically, and under controlled conditions. This evidence was obtained in connection with a study of the inflammatory edema produced by paraphenylenediamin. Although the study of the

*From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco, Calif.

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mechanism of this edema is still in progress, the results with the antiphlogistic agents employed were definite enough to warrant presentation at this time and independently of our other work. Briefly summarized, our results with salicylate and neocinchophen in the treatment of this experimental condition were entirely negative. Those with cinchophen were partly negative and partly positive, giving the impression of a beneficial action in some cases. However, when the apparently positive results were properly considered, they were found to be negative also, indicating that the apparent beneficial effects from these agents reported by others are susceptible of a different interpretation.

METHODS

Edema of the head and neck was produced by the hypodermic administration of freshly dissolved paraphenylenediamin hydrochloride* to rabbits, using a dosage of 0.19 gm. per kilo. With this dosage the majority of rabbits respond with a peculiar specific edema of the head involving the tongue, lips and orbital tissues, and a pouchy swelling in the submaxillary region and of the cervical skin. The tongue is large and thick and protrudes from the mouth, the nose is broad, the cheeks are full and the eyes give the appearance of exophthalmos. There are usually a marked chemosis and considerable salivation. The effects appear anywhere from one hour to three hours after injection, and last throughout the second day if the animal survives. With a dosage of 0.19 to 0.2 gm. per kilo subcutaneously the animals usually die at the end of 6 to 8 hours, but with smaller doses they may survive for 3 to 4 days and occasionally recover completely. The majority of animals die eventually, irrespective of dosage, and death is due presumably to intercurrent disease as a result of lowered resistance imposed by the action of the para. Cats and dogs respond similarly to rabbits; in cats more marked swelling of the vocal cords supervenes, and this leads to asphyxia with death rather rapidly. The cause of death in rabbits is, in part, also due to asphyxia from obstruction by swellings in the pharynx and larynx, but, in part, it is due to circulatory failure from the drug. This has been described in a previous study by one of us.[8]

Therefore, in order to reduce the effects of asphyxia from respiratory obstruction and prolong the life of the rabbits, all the animals in this study were previously tracheotomized. At the same time blood was secured by cardiac puncture through the chest wall for evidences of changes in blood concentration as indicated by changes in hemoglobin and total solid content of the blood. Hemoglobin was estimated colorimetrically by the carbon monoxide method of Palmer,[9] using 0.2 to 0.3 c.c. of blood diluted to 20 to 30 c.c. (1 per cent). The first specimen before administration of the para was taken as the control, and given the value of 100 per cent. Subsequent values, therefore, represent relative concentrations of hemoglobin. For the total solids, 0.5 c.c. of blood was dried in a tared porcelain crucible at 110° until the weight was constant; this served as a check on the results with hemoglobin. A total of not more than 0.8 c.c. of blood was withdrawn for these estimations. Withdrawal of larger quantities caused too much blood dilution, and prevented the recognition of the usual change of an increase in blood concentration produced by the para. The escape of so much fluid from the circulation into the tissues during the edema would naturally lead to an increase in concentration of the fixed and nondiffusible elements of the blood. The cause of this increase in concentration is thought to be due to an increase in vascular permeability produced by the para, but why this should occur preeminently in the head and neck without demonstrable edema and exudates in other regions of the body is not known. The discussion of this phase of our study pertains to the original object of our problem, and is waived in the present connection. Here the phenomenon of increased blood concentration is used solely as an objective criterion of the edema process, although careful notes of gross anatomic and postmortem changes were also made in each animal. It was

*Hereafter the term "para" in place of "paraphenylenediamin hydrochloride" is used throughout the text.

especially valuable in determining the tendency to edema in cases of doubtful swelling (by inspection). Hence, if this phenomenon, or the gross edema, or both, were antagonized or prevented without other complications by the agents that were tested, it was regarded as evidence of antiphlogistic action. Barbour[10] and coworkers have shown that the administration of sodium salicylate and acetylsalicylic acid to fevered dogs and human individuals causes blood dilution. Although this change appears to be connected with the antipyretic qualities of these agents, it might be due to other factors, for instance, circulatory depression, which we believe to be concerned in the blood dilutions in certain of the experiments of this study and to be discussed later. The body temperature of the rabbits in the present experiments was not observed, but in a previous study it was shown to be lowered generally by para, except during the convulsant stage when it was raised. Therefore, the changes in blood concentration reported indicate not only the influence of the various antiphlogistic agents on the edema process of para, but also the diluting effects of these agents on concentrated blood independently of fever.

The results that were obtained are presented in the form of curves in Figure 1. These illustrate the effects of the different agents on blood concentration. Other data are presented in Table I. At least four rabbits were used with each agent. The experiments were planned in such a way as to exhibit the action of the antiphlogistic agents most favorably. That is, maximal doses corresponding to the clinical "toxic" doses used in the treatment of rheumatic fever were used. These were administered subcutaneously and gastrically as single and divided doses.

Sodium salicylate was administered as a 10 per cent aqueous solution subcutaneously. Two rabbits received 0.15 gm. per kilo, and one received 0.2 gm. per kilo in single doses, and one was given 0.2 gm. per kilo in 4 divided doses. The cinchophen was dissolved in a little 10 per cent sodium hydroxide and then was administered intragastrically through a small stomach tube and bulb, washing down the remnants with a little water. The dosage ranged from 0.1 to 0.4 gm. per kilo. Neocinchophen was administered intragastrically in the form of a suspension in water. The dosage ranged from 0.25 and 0.5 gm. per kilo. In view of the marked insolubility of neocinchophen, the high dosage of 0.5 gm. per kilo was tried in order to provide an adequate quantity for absorption. However, the result was the same as with smaller doses. The para was injected after sufficient time interval had elapsed for the development of the actions of the antiphlogistic agents. With salicylate this amounted to from 45 minutes to 1 hour; with cinchophen, from 1½ to 3 hours, and with neocinchophen, from 2 to 3 hours.

BLOOD CONCENTRATION

It is seen that the blood concentration, as indicated by the percentage increases in hemoglobin and total solids (Fig. 1.), was promptly and definitely increased by the injection of para alone as control. These increases in concentration, though variable, were not prevented or antagonized by the administration of salicylate to 3 out of 4 rabbits, of cinchophen to 3 out of 10 rabbits and of neocinchophen to the 4 rabbits that were used. Rabbit 35, receiving 0.2 gm. per kilo of salicylate, showed a decrease in concentration to 88.9 per cent, of hemoglobin and to 90 per cent of total solids. Rabbits 40, 49, 50, 52, 53, 57 and 58, which received the highest doses of cinchophen, namely, from 0.15 to 0.4 gm. per kilo (Table I), also showed considerable decreases in concentrations of hemoglobin (94.3 to 75.2 per cent) and total solids (93.8 to 78.5 per cent; one rabbit, 58, showed 102.5 per cent). (The values for these animals are given in the text because they are similar and could not be used satisfactorily for the construction of legible curves in the accompanying figure). Grossly, these rabbits, which received cinchophen, and showed diminished blood concentrations, showed moderate,

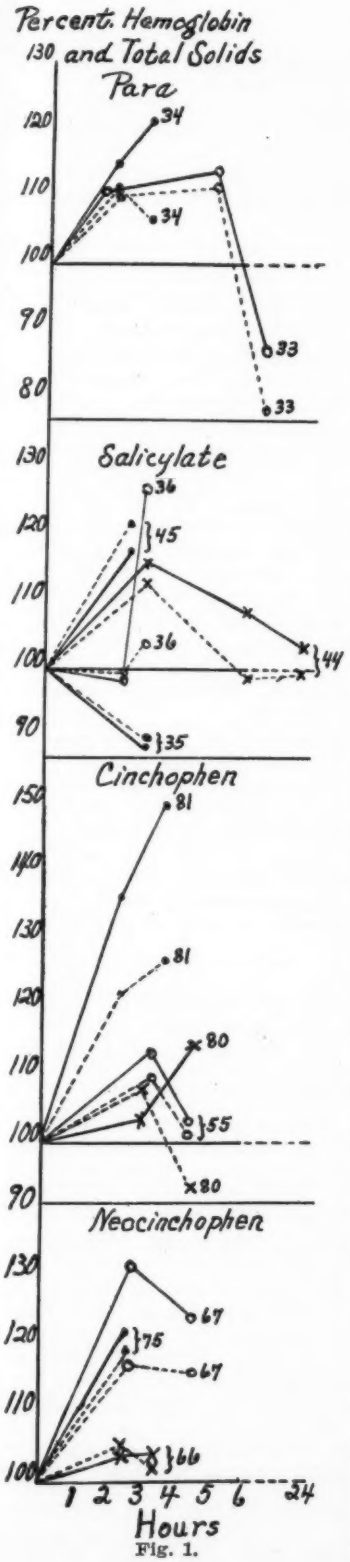

Fig. 1.

mild or no edema of the head and neck, while the single rabbit receiving salicylate and showing diminished blood concentration showed very marked edema of the head and neck. This at once suggests that the apparent antiedema and antiblood concentration effects of cinchophen in these seven rabbits were accidental and not due to the cinchophen. This was supported by the facts that similar effects were encountered with para alone, and with other agents, which are being employed in our study of the para edema in the same way as the antiphlogistic agents in this study. For instance, rabbit 42, which received quinin hydrochloride, showed a decrease of hemoglobin to 81 per cent, and of total solids to 88 per cent at time of death, and rabbit 51, which received antipyrin, showed a decrease in hemoglobin to 94 per cent, and of total solids to 88 per cent. In the animals injected with para alone, more blood was withdrawn from the heart than in the animals here reported, but the cause for these decreases in blood concentration is probably the same. It occurred invariably shortly before or at time of death, and at a time when the circulation was impaired or had collapsed. Impending collapse could frequently be recognized from the sluggish and incomplete rise of the blood into the pipette used for cardiac puncture. This meant a weakened intraventricular pressure due to cardiac depression produced by the para, and was accentuated by the salicylate and cinchophen. It had been shown previously by one of us that para, in large doses, depresses the heart muscle directly and all coal tar derivatives (salicylate and cinchophen) are notorious for this. Hence, it is clear that the apparent antiedema and antiblood concentration effects of the cinchophen in the 7 rabbits, and of salicylate in the single rabbit in which they occurred, are not peculiar to or specific for these drugs, but that they occur

Fig. 1.—Effects of paraphenylenediamin hydrochloride, and of sodium salicylate, cinchophen and neocinchophen in rabbits receiving paraphenylenediamin hydrochloride, on the percentage concentration of hemoglobin and total solids of the blood. "Para" refers to paraphenylenediamin hydrochloride injected subcutaneously in the dose of 0.19 gm. per kilo throughout. For dosage of the remaining agents, Table I should be consulted. The figures opposite the curves refer to numbers of the experiments (rabbits). Continuous lines refer to hemoglobin, and broken lines to total solids.

TABLE I

EFFECTS OF PARAPHENYLENEDIAMIN HYDROCHLORIDE, AND OF ANTIPHLOGISTIC AGENTS IN RABBITS RECEIVING PARAPHENYLENEDIAMIN HYDROCHLORIDE SUBCUTANEOUSLY (0.19 GM. PER KILO)

NUMBER AND WEIGHT* OF ANIMAL	DOSE OF ANTIPHLOGISTIC AGENT ADMINISTERED (GM. PER KILO)	EDEMA AND TIME OF APPEARANCE** + = present − = absent (hours)	TIME OF DEATH† (HOURS)	REMARKS, AND CHANGES AT AUTOPSY
PARAPHENYLENEDIAMIN HYDROCHLORIDE (0.19 GM. PER KILO)				
31 (1.6)		+ 2	5½	Large areas of edema of head, neck, tongue with chemosis and exophthalmos.
33 (2.2)		+ 1½	15	Marked edema of face and neck, with exophthalmos and chemosis, tongue swollen and protruding from mouth.
34 (1.8)		+ 1½	3	Definite edema of head and neck. Tongue protruding, exophthalmos and chemosis.
SODIUM SALICYLATE				
35 (1.7)	0.2 (H)	+ 2	3¼	Blood sample obtained at time of death. Marked edema of head and neck. Exophthalmos and chemosis with protrusion of tongue. Hemoglobin 88.9 per cent; total solids, 90 per cent. Blood diluted.
36 (2.5)	0.15 (H)	+ 2	3	Very marked edema of head and neck chemosis, exophthalmos and protrusion of tongue present. Respiration rapid. Heart depressed. Vocal cords edematous, closing of larynx.
44 (2.3)	0.15 (H)	+ 3	survived	Moderate edema of head and neck observed. Tongue swollen and protruding. Exophthalmos present. Large area of edema at site of injection of para.
45 (2.4)	0.2 (H)	+ 1	2½	Salicylate administered in 4 divided doses. Marked edema of head and neck with exophthalmos, chemosis, protruding tongue. Larynx closed by edema of vocal cords. Edema at site of injection of para.
CINCHOPHEN				
40 (1.3)	0.4	+ 1	1	Some chemosis and thickening of tongue at autopsy. No other gross edema. Hemoglobin 82.6 per cent, total solids, 93.8 per cent.
49 (1.8)	0.3	+ 3½	3½	Moderate edema of neck at autopsy with swollen tongue and marked edema of vocal cords. Abdomen contained 35 c.c. of gelatinous exudate. Hemoglobin, 86.6 per cent. Total solids, 78.5 per cent.
50 (2.0)	0.3	+ 2¼	2¼	Definite edema of vocal cords. Slight edema of neck and tongue. Hemoglobin, 72.2 per cent; total solids, 81.1 per cent.
52 (1.8)	0.25	+ (?)	1½	Questionable edema of neck at autopsy. 10 c.c. free fluid in abdomen. No change elsewhere except edema locally at site of injection of para. Hemoglobin, 83.7 per cent, total solids, 84.9 per cent.
53 (1.8)	0.25	+ 1½	1½	Very slight edema of neck and moderate edema of vocal cords. Hemoglobin, 82.6 per cent; total solids 81.5 per cent.
55 (2.1)	0.15	+ 2½	4¼	Marked edema of head and neck, and vocal cords.

*Kilos.
**Hours.
†After administration of paraphenylenediamin hydrochloride. (H) refers to hypodermic injection; the remaining administrations were gastric.

TABLE I—CONT'D

EFFECTS OF PARAPHENYLENEDIAMIN HYDROCHLORIDE AND OF ANTIPHLOGISTIC AGENTS IN RABBITS RECEIVING PARAPHENYLENEDIAMIN HYDROCHLORIDE

NUMBER AND WEIGHT* OF ANIMAL	DOSE OF ANTIPHLOGISTIC AGENT ADMINISTERED (GM. PER KILO)	EDEMA AND TIME OF APPEARANCE** + = present − = absent (hours)	TIME OF DEATH (HOURS)	REMARKS, AND CHANGES AT AUTOPSY
		CINCHOPHEN (CONTINUED)		
57 (2.5)	0.15	−	2	Nothing abnormal at autopsy. Hemoglobin, 86.2 per cent; total solids, 92.2 per cent.
58 (2.4)	0.15	+ 2	2	Slight edema of nose and tongue. Vocal cords edematous. Hemoglobin, 94.3 per cent; total solids, 102.5 per cent.
80 (2.0)	0.1	+ 2	4½	Very marked edema of head and neck. Blood concentration increased. Exophthalmos, chemosis and protrusion of tongue present.
81 (2.1)	0.1	+ 1½	3¾	Very marked edema of head and neck with exophthalmos, chemosis, and protruding tongue, 15 c.c. gelatinous exudate in the pleural cavities.
		NEOCINCHOPHEN		
66 (2.1)	0.25	+ 2½	3½	Marked edema of head and neck, with exophthalmos, protruding tongue. Vocal cords edematous.
67 (2.1)	0.4	+ 2¾	4½	Very marked edema of head and neck, with exophthalmos and chemosis, death from pulmonary edema. Larynx closed by edema of vocal cords.
70 (2.3)	0.4	+ 1½	1½	Tongue swollen and protruding; exophthalmos and chemosis present. Vocal cords edematous. No blood secured.
75 (2.1)	0.5	+ 2	2½	Very marked edema of head and neck. Exophthalmos and edema of vocal cords pronounced. Rabbit had survived a previous experiment (61) without showing edema.

*Kilos.
**Hours.
†After administration of paraphenylenediamin hydrochloride. (H) refers to hypodermic injection; the remaining administrations were gastric.

with para alone, that they may be connected with the toxicity of cinchophen and salicylate, and finally that they go parallel with circulatory collapse in any case. When increases in blood concentration were demonstrable they fortified the results observed by inspection.

GROSS CHANGES

The gross changes in the majority of rabbits used in this study were unmistakable, and, therefore, convincing proof of the actions of the agents tried was obtained. Qualitatively, the effects were exactly the same as with para alone. These were detailed in the fore part of the paper and need not be repeated again. They were observed in all of the 4 rabbits receiving salicylate, in 6 out of the 10 receiving cinchophen and in all of the 4 receiving neocinchophen.

The time of onset of the edema was from ½ hour to 3 hours (median 1½ hours) in the salicylated rabbits; from 1 hour to 3½ hours (median 2 hours) in the cinchophen treated rabbits; and from 1½ to 2¾ hours (median 2¼ hours) in the neocinchophen treated rabbits. The time of onset in rabbits receiving para alone was from 1½ to 2 hours (median 1½ hours). In other words, the antiphlogistic agents tended to retard the time of onset, if only the median values are considered. But, if the experiments are considered individually, the tendency to retardation is not so apparent, since the majority of results fall within the range of time of onset from para alone. Hence, it is indicated that the antiphlogistic agents exerted no definite beneficial influence in this direction.

MORTALITY

This was 100 per cent for animals receiving para alone, para with cinchophen and para with neocinchophen, and 75 per cent in the 4 animals receiving para with salicylate. This high mortality was due to the relatively high dosage of para used and which is necessary for the successful production of edema in the majority of animals. The single recovery in the salicylate treated group was accidental, since, in our experience, one out of any four rabbits taken at random is apt to survive the dosage of 0.19 gm. per kilo of para alone, but this proportion of survivals is not necessarily present with a much greater number of animals used, and, in fact, tends to be reduced. The results show conclusively that treatment of the rabbits with the antiphlogistic agents did not reduce or prevent the mortality from para.

As to time of death (after administration of para) the results with the antiphlogistic agents were even less gratifying than the percentage mortality. That is, although the death of all the animals was expected eventually, in view of the high dosage of para used, yet, if there had been a uniform retardation in time by the antiphlogistic agents this would have been regarded as a beneficial influence. However, the opposite was the case. That is, death was hastened when these agents were used together with para, as compared with para alone. The results in Table I indicate that the time of death in rabbits receiving para alone was from 3 to 15 hours (median 5½ hours); in those receiving para with salicylate, 0 to 3¼ hours (median 2¾ hours); in those receiving para with cinchophen, 1 hour to 4½ hours, (median 2⅛ hours); and in those receiving para with neocinchophen, 1½ to 2¾ hours (median 2¼ hours). As indicated by the median values, the duration of life was shortened about one-half in the animals receiving the different antiphlogistic agents together with para as compared with those receiving para alone. The high values of 5½ and 15 hours for time of death in 2 out of 3 rabbits receiving para alone was not attained by a single one of the larger groups of rabbits receiving para together with antiphlogistic agents. In other words, time of death in these animals was hastened, and, therefore, the antiphlogistic agents were detrimental. This detrimental tendency was more pronounced with cinchophen than with salicylate and neocinchophen.

AUTOPSY

Except for a somewhat greater tendency to swelling of the larynx and vocal cords in the rabbits receiving salicylate, and neocinchophen together with para, the changes at autopsy were practically identical with those receiving para alone. These changes have been described previously, and also in the fore part of this paper, and need not be repeated. Of the animals receiving cinchophen together with para, one (rabbit 57) showed nothing abnormal at autopsy, one (rabbit 52) showed some edema at the site of injection and a questionable edema of the neck only, and the remaining 8 showed variable degrees of edema in the usual regions influenced by para. Three of the animals showed some additional and unusual changes, namely, gelatinous exudates in the peritoneum (rabbits 49 and 52) and pleura (rabbit 81). As far as cinchophen is concerned, the tendency of the changes at autopsy in the majority of animals agrees, in general, with that of the changes in other directions as described above. That is, cinchophen was consistently more deleterious than the salicylate and cinchophen, and tended to aggravate and extend the effects of para.

DISCUSSION

Taking everything into consideration, the results of these experiments do not indicate that salicylate, cinchophen and neocinchophen ameliorated the edema and blood concentration effects of para in any way whatsoever, and, therefore, as antiphlogistics under the conditions they were negligible. This conclusion agrees completely with that of Lacquer and Magnus[7] regarding the antiphlogistic actions of salicylate and cinchophen in the pulmonary edema produced by phosgene in cats, and with that of Heubner and Gildemeister[6] for the antichemosis action of cinchophen.

Cumulatively, the experimental evidence indicates that these drugs do not exert a beneficial influence on the inflammatory changes, including the swelling, edema and exudate of joints, in acute rheumatic fever through a direct, organotropic influence, unless the inflammatory changes of joints in acute rheumatic fever are different from similar pathologic changes in other regions produced experimentally (para, etc.), but there is no reason to assume that this is the case. Because of our ignorance of the etiology of acute rheumatic fever, it is not yet possible to produce a satisfactory experimental arthritis and test this proposition directly, and, therefore, the etiotropic action must be waived for the present also. In the present state of our knowledge, the most satisfactory explanation of the mechanism of action of salicylate, cinchophen and similar drugs in rheumatic fever, including the antiphlogistic action, is the symptomatic, that is, relief in this condition appears to be mediated through the antipyresis and analgesia produced by these agents, which act rather efficiently. Evidence for this notion was reported previously.[11]

CONCLUSIONS

1. Experimental edema of the head and neck produced by the systemic administration of paraphenylenediamin in rabbits was not beneficially influenced by previous and simultaneous treatment of the animals with sodium salicylate, cinchophen and neocinchophen in large doses corresponding to the clinical "toxic" dosage used in the treatment of acute rheumatic fever.

2. If anything, these drugs tended to aggravate this pathologic condition. All hastened death, but especially cinchophen, which, in addition, favored the occurrence of peritoneal and pleural exudates. The increase in blood concentration, which accompanied the edema of paraphenylenediamin, remained unchanged by the administration of salicylate, cinchophen and neocinchophen, except shortly preceding, or at time of, death when it was lowered, but this also occurred at death with paraphenylenediamin alone, and, therefore, cannot be attributed to any peculiarity or specificity of these drugs.

3. Therefore, taking everything into consideration, the alleged antiphlogistic actions of salicylate, cinchophen and neocinchophen, when tested under these conditions, were found to be wanting, and so far as salicylate and cinchophen are concerned, this agrees with the negative results of other investigators, who treated other kinds of edemas.

4. The beneficial therapeutic effects of these drugs in rheumatic fever appear to be produced neither through etiotropic nor organotropic, but rather through symptomatic action, the benefits being mediated through antipyresis and analgesia.

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STUDIES ON THE PHARMACOLOGY OF SODIUM CITRATE*
I. CIRCULATION

By George R. Love, M.D., Chicago, Ill.

ALTHOUGH sodium citrate is introduced into the blood stream quite frequently in transfusions very little study has been devoted to its action upon circulation. Since these studies were undertaken, articles on the action of sodium citrate by Salant and coworkers have been published. While our work on sodium citrate is of a similar nature we have made further observations which are of interest. In instances where our experiments confirm previous observations the general results will be presented rather than specific data.

SODIUM CITRATE

Several brands of "U. S. P." and "C. P." salts were used in the investigation. Although the animals showed some variation in response to citrate we found no significant difference in action of the several salts upon any individual animal. To avoid large volume of injection the concentration of the solution injected was ten per cent.

BLOOD PRESSURE

That sodium citrate is a direct cardiac depressant has been demonstrated by Gros,[1] Salant and Hecht,[2] and Salant and Kleitman.[3] The latter observed that although the heart was markedly depressed (as recorded by a myocardiograph) the blood pressure sometimes remained unaffected or even rose after the injection. This phenomenon they attribute to prompt vasomotor adjustment. Salant and Kleitman observed also that the blood pressure was not constant in dogs; doses of 50 to 80 mg. per kilo in some animals produced a rise, in others a fall of pressure. These differences they attribute to changes in the irritability of the vagus mechanism. They conclude that small and medium doses of sodium citrate stimulate while larger doses depress the cardio-inhibitory center.

All of our experiments were made upon dogs under light ether anesthesia. Blood pressure was recorded by a mercury manometer. In our myocardiograph experiments either the adjustable tambour or heart lever method was used[4]. The results confirm the observations of Salant and Kleitman in that the heart may be depressed with either a rise or fall or no appreciable change in the blood pressure. This action occurs after section of the vagi, after atropine, and often with no appreciable change of heart rate. By further experiments we were convinced that sodium citrate acts upon mechanisms controlling blood pressure other than those previously reported.

*From the Departments of Pharmacology of the University of Illinois, College of Medicine, and the General Medical College of Chicago.
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DOSE AND RATE OF INJECTION

When due allowances are made for individual animals and changes in depth of anesthesia the following data are fairly constant. Doses of 50 mg. per kilo given intravenously to small or medium size dogs over a period of one minute will depress the heart and depress the blood pressure at first, but will raise the blood pressure at the end of the minute to normal or a few millimeters above. If 100 mg. per kilo be given under the same conditions the blood pressure falls during the first part of the minute and rises

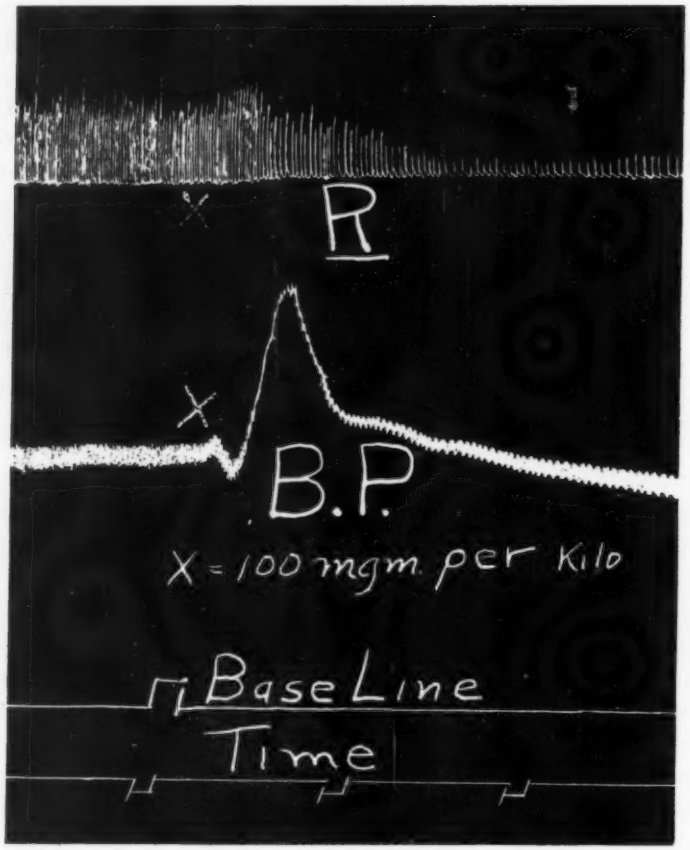

Fig. 1.—Respiration and blood pressure. Injection of 100 mg. per kilo of sodium citrate. Time in minutes.

above normal in the latter part. Thus a double curve is produced in which a depressor action is followed by a more marked pressor action. If 100 mg. per kilo be given at the rate of 10 mg. per kilo per second there appears a sharp fall followed by an equally sharp rise of blood pressure which lasts from one-half to four minutes. The rise of blood pressure varies from 10 to 100 per cent greater than the original level. Fig. 1 represents such a graph. On some animals after the first or second injection of sodium citrate the pressor action comes on so suddenly that the blood pressure rises from the beginning. Fig. 2 represents such an experiment. The conclusion, there-

fore, seems reasonable that vasomotor compensation is only a factor in the rise of blood pressure following the injection of sodium citrate, and the chief cause of the rise of pressure is a primary pressor stimulation.

An analysis of the blood pressure tracing as in Fig. 1 on a rapidly moving drum in conjunction with a myocardiograph record shows the following: During the fall of blood pressure the heart is depressed, and the rate is decreased. During the rapid rise of blood pressure the heart appears to be actually stimulated, particularly the auricles, and the rate is markedly increased. Fig. 3 illustrates this action on a slow revolving drum. Fig. 4 illustrates the comparative action upon the left ventricle and right auricle.

Since sodium citrate is a direct cardiac depressant when injected more slowly, it is not reasonable to suppose that the increase of heart rate and

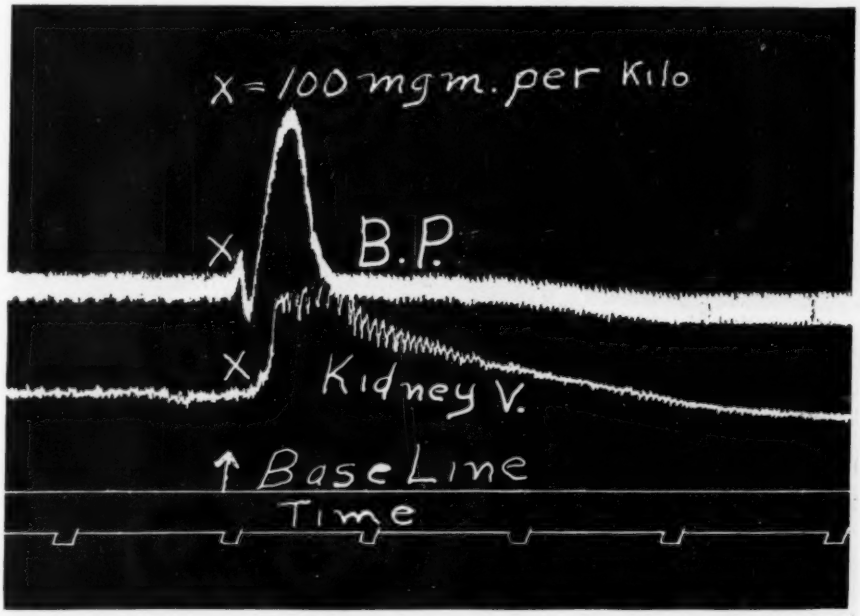

Fig. 2.—Blood pressure and kidney volume. At x a dose of 100 mg. per kilo was injected rapidly. Time in minutes.

action when injected rapidly are due to direct stimulation of the muscle. Since the pressor action occurs after atropine and is most marked in the auricles it appears to be due to stimulation of the accelerator nervous mechanism.

It is obvious that the increased heart rate and action, as illustrated in Fig. 1, do not explain the fact that it is possible by slow injections to maintain blood pressure and depress the heart simultaneously with no appreciable change in rate.

MEDULLARY CENTERS

During the primary fall of pressure (Fig. 1) the rate was often slower. After repeated injections the heart rate was increased. This was observed by Salant and Kleitman who attributed the slowing of the rate to vagus

stimulation and the increase of rate after repeated injections to depression of the cardio-inhibitor center.

After section of the vagi or after 2 mg. atropine the first injections of sodium citrate generally caused a perceptible slowing of the heart. Since the slowing was often of short duration it was difficult by our methods to determine to what extent the action was upon the vagus or heart muscle;

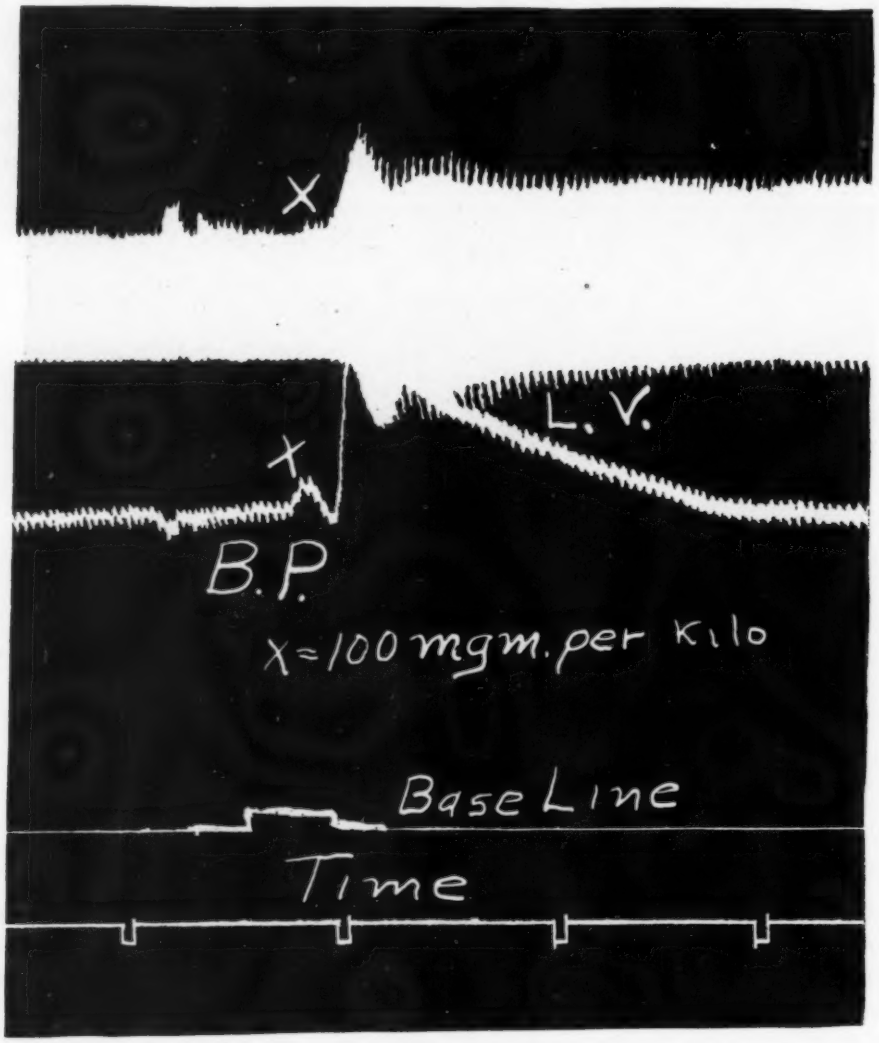

Fig. 3.—Left ventricle, recorded by adjustable tambour method and blood pressure. Dog 6 kilo. Injection of 100 mg. per kilo of sodium citrate. Time in minutes.

each animal had to be a control within itself. In a number of experiments the pulse pressure (stroke of manometer lever) was markedly increased during the fall of blood pressure. After section of the vagi or after atropine the rate was still decreased but the pulse pressure either remained normal or was decreased. Since the effects varied with each injection the comparative decrease of rate and fall of blood pressure before and after atropine was

of no quantitative value. However, after repeated injections of the salt until the heart rate was distinctly faster, aconite still slowed the pulse. This would indicate that the center was not paralyzed or seriously impaired. Furthermore, after section of the vagi repeated injections of sodium citrate still accelerate the heart. These facts indicate that the heart was temporarily slowed by direct action upon the heart and by vagus stimulation. The increase of heart rate during the rise of blood pressure and after repeated injections was due chiefly to cardio-accelerator stimulation.

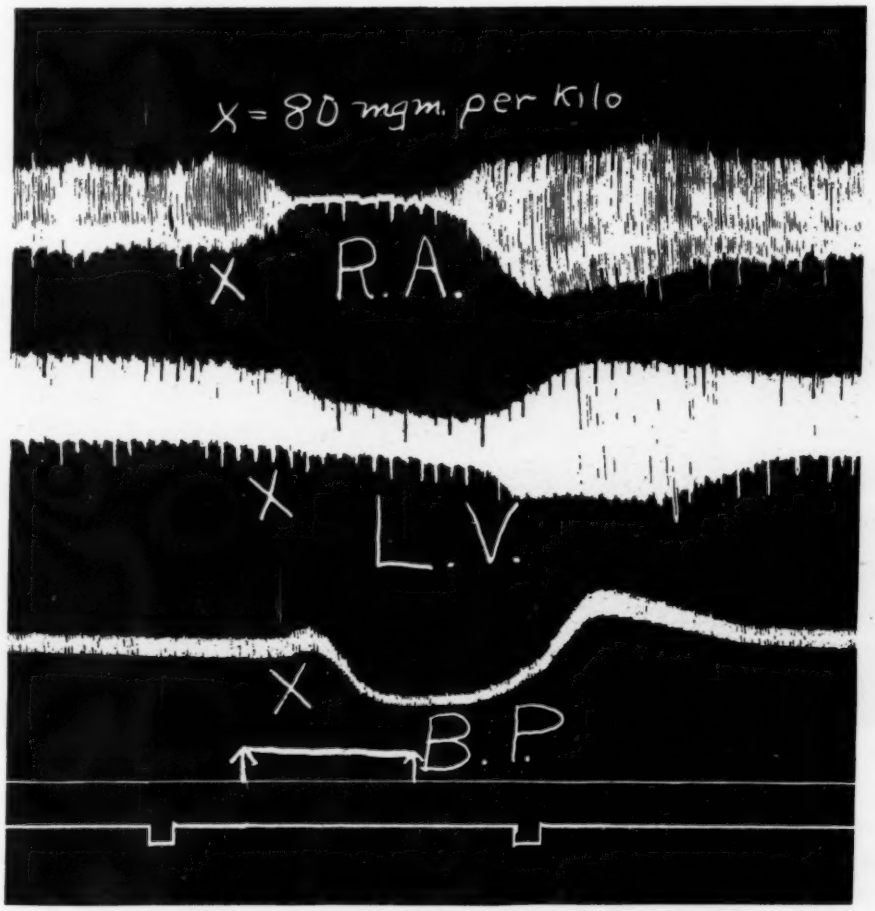

Fig. 4.—Right auricle and left ventricle by heart lever method. Eighty milligrams per kilo of sodium citrate injected. Time in minutes.

Injections of 1 c.c. of 3.0 per cent (practically isotonic) sodium citrate into the fourth ventricle indicate that the vasomotor center is stimulated. The technic of such experiments was to make the puncture, withdraw 1 c.c. fluid, and inject 1 c.c. of the citrate solution. The needle was left open immediately after the injection to prevent pressure stimulation. Such experiments are not conclusive for reasons quite obvious. The needle puncture itself often causes vagus stimulation. Furthermore, the action of a drug applied locally to the medulla may not be related to the action that drug

may have when introduced through the blood stream. Fig. 5 illustrates the effect of 1 c.c. of 3.0 per cent sodium citrate injected into the fourth ventricle. In this experiment a part of the vagus stimulation may be mechanical. The pressor action however is undoubtedly due to the action of the salt since it occurs only when the salt is injected and persists even when the puncture needle is left open and in position.

Experiments in which the sodium citrate was injected into the carotid artery gave more reliable data as to central action. The technic of such experiments was to take the blood pressure from a femoral artery, inject 1 to 4 c.c. of 3.0 per cent sodium citrate with a fine hypodermic needle into a carotid artery. The effect on the blood pressure varies according to the dose and the rate of injection. A dose of 30 mg. per kilo injected in one

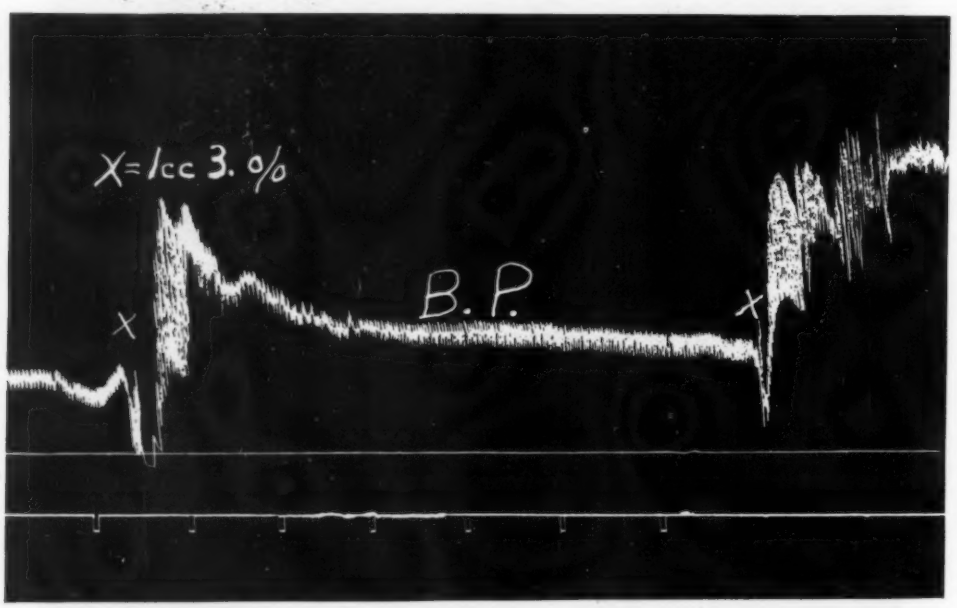

Fig. 5.—Blood pressure and fourth ventricle injections. Two injections of 1 c.c. of 3.0 per cent citrate were made at the points x. Time in minutes.

minute produces a pressor action with usually a decrease of heart rate. Large doses produce a pressor action followed by a depressor action. The depressor action is due to the citrate returning to the heart in sufficient amounts to impair its function. This is easily demonstrated by the myocardiograph. After 2 mg. atropine, carotid injections usually gave an increase of heart rate, indicating that the cardio-accelerator center is stimulated in a lesser degree. Fig 6 illustrates the stimulating action upon the vasomotor centers by injecting sodium citrate into a carotid artery. The respiration is at first stimulated by small doses and depressed by larger doses following carotid injections.

The action upon the vasomotor center explains in part at least the phenomenon that with sodium citrate it is possible to depress the heart and maintain blood pressure with no appreciable change of heart rate.

PERIPHERAL ACTION

The study of the peripheral action of sodium citrate in relation to blood pressure by means of plethysmographs is not satisfactory because of the double curve of blood pressure. The kidney volume, however, often shows evidence of some active dilatation of the vessels. In Fig. 2 there is a temporary downward dip in the blood pressure following the injection of citrate while the oncometer tracing continues to rise. In this particular experiment

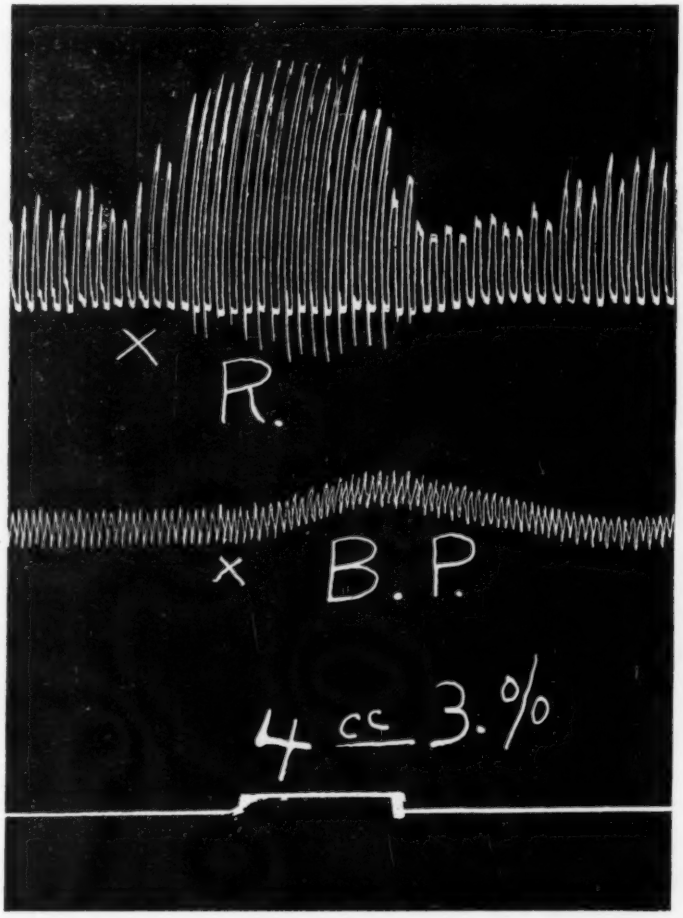

Fig. 6.—Respiration and blood pressure. At x injected 4 c.c. of 3.0 per cent into a carotid artery. Dog 4 kilo.

the oncometer was sufficiently sensitive to record the blood pressure dip if the volume curve was merely following the blood pressure. Also the kidney volume remains increased even after the blood pressure falls to its original level. Perfusion of the kidney with Locke's solution at 37° C. also shows an active dilatation of the kidney vessels by sodium citrate. The perfusions were made under a pressure of 120 mm. Hg., and the sodium citrate (2 c.c. of 3 per cent solution) was introduced by a T cannula near the renal artery cannula. Such a dose of citrate gave a 10 to 30 per cent increase of out-

flow which lasted from three to ten minutes. However, in some animals the kidney oncometer tracing followed the blood pressure down but did not follow the rise of pressure proportionately. Fig. 7 illustrates this action, which indeed may be a compensatory or central action.

The oncometers for the limbs and intestines were not so sensitive as the kidney rubber diaphragm oncometers. So far as we were able to determine, the plethysmograph tracings of the intestines and the extremities follow the blood pressure curve.

Plethysmograph tracings of the upper respiratory passages and the buccal cavity follow the blood pressure down but do not rise proportionately with the pressor stimulation. The volume is often less even when the blood pressure rises 50 to 100 per cent above the original level. The technic employed was a modification of the method described by Tschalussow.[5] The

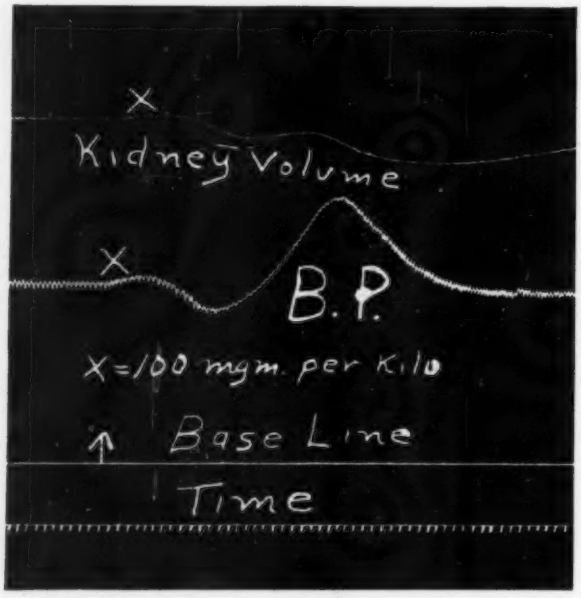

Fig. 7.—Kidney volume and blood pressure. Dog 9 kilo. At x 100 mg. per kilo. Time in seconds.

upper trachea and esophagus were tied off. The upper lips were drawn over the lower lips, stitched, and sealed with lanolin. The entire cavity was filled with mineral oil (37°C) introduced through a nasal passage. A cannula connected to the recording tambour was placed in one nasal passage and sealed in with oiled cotton. The other nasal opening was likewise closed with oiled cotton, lanolin, etc.

EXCISED ARTERIES

Excised carotid and internal iliac arterial rings were tested according to the method described by Macht.[6] The results were negative. Also the application of 3.0 per cent sodium citrate to a wound or to the conjunctiva gave no evidence of vasoconstriction.

SITE OF ACTION UPON THE ACCELERATOR MECHANISM OF THE HEART

After bilateral removal of both stellate and inferior cervical ganglia the pressor action was still present. However the pressor action is eliminated by suitable doses of ergot. The routine of such experiments was as follows: Sodium citrate, 100 mg. per kilo was injected and followed later by 1 c.c. of epinephrine (1-10,000). Fluid extract of ergot (the alcohol of which had been previously evaporated to a low percentage*) was injected in 1 c.c. per kilo doses until epinephrine lowered the blood pressure. (The amount of ergot necessary, in terms of the fluid extract, varied with the preparation. As a rule 4 to 5 c.c. per kilo were sufficient, but with one preparation 11 c.c. per kilo were necessary.) The sodium citrate injection was then repeated with exactly the same dose and rate of injection as before ergot.† Fig. 8 illustrates the action of sodium citrate upon blood pressure before and after

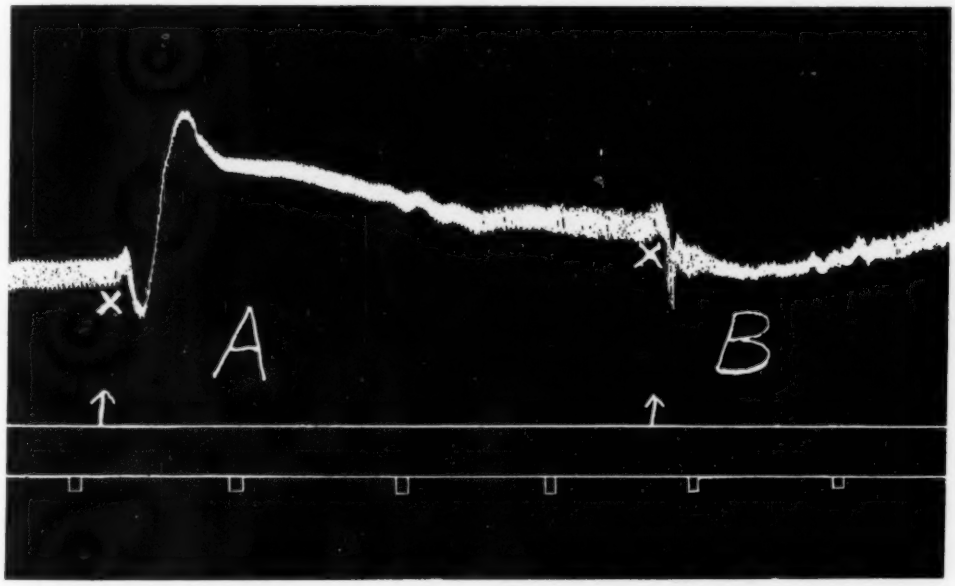

Fig. 8.—Blood pressure on 5 kilo dog. *A*, sodium citrate 100 mg. per kilo before ergot; *B*, same after ergot.

ergot. After ergot the depressor action of citrate was more prolonged, during which there was a marked slowing of the heart. This was due to direct action upon the heart muscle and vagus stimulation, and was overshadowed before ergot by the stimulation of the accelerator nerves.

DISCUSSION

While our results on blood pressure appear at first to be contradictory in some respects to those of Salant and Kleitman,[3] the experiments are not comparable. Their experiments were so arranged as to bring out the depressor action upon the heart and we can confirm these results. Our experi-

*During the course of these experiments ergotoxin was not available.

†Adrenalin still accelerated the heart after ergot, although the blood pressure was lowered. The adrenalin acceleration was, however, distinctly more marked before than after ergot.

ments deal chiefly with the pressor action of citrate. The smaller doses of citrate stimulate the vasomotor center and depress the heart muscle. The blood pressure in such cases will depend upon which action predominates. The rapid injection of the smaller doses favors a predominance of cardiac depression. With the rapid injection of the larger doses stimulation of the endings of the accelerator nerves of the heart becomes quite marked and together with the central vasomotor action produces a pressor action upon blood pressure. This pressor action is of short duration, but sodium citrate disappears equally rapidly from the blood stream.[7] This is not a volume effect because rapid injection of even a greater volume of physiologic saline produced a comparably slight rise of pressure. While it seems that the vessels of the kidney may undergo some active dilatation, yet the action persists only a very short time, and the dose necessary to produce this effect renders the action of no therapeutic interest. The medullary centers are stimulated by suitable doses. The action on the cardio-accelerator center is overshadowed normally by the inhibitors, but is easily demonstrable after atropine or section of the vagi.

The problems involved in the decrease of volume of the upper respiratory and buccal membranes were not investigated because the action was not sufficiently striking to warrant a further sacrifice of animals.

CONCLUSIONS

1. The heart muscle of dogs is depressed by doses of 30 mg. sodium citrate per kilo or more.

2. The vasomotor center is stimulated by suitable doses of citrate.

3. The cardio-inhibitor and cardio-accelerator centers are stimulated.

4. The endings of the accelerator nerves to the heart are markedly stimulated by large doses of citrate.

5. There is some evidence that large doses cause active dilatation of the vessels of the kidney.

6. Plethysmograph tracings of the extremities and intestines show no peripheral action upon blood vessels.

7. Sodium citrate, in the dose used and at the rate injected in blood transfusions, produces no effect upon circulation which would militate against its use as an anticoagulant.

The author wishes to acknowledge his indebtedness to Dr. Huph McGuigan for suggestions and criticism of this work.

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FURTHER NOTES ON D'HERELLE'S PHENOMENON*

By Earl B. McKinley, M.D., Dallas, Texas

D'HERELLE[1] has reported a variety of sources from which he has isolated bacteriophages. In the excreta of healthy men, horses, fowls and other divers animals including monkeys, cats, cattle, buffaloes, swine, rabbits and goats he has been able to demonstrate their presence. In certain cases he states bacteriophages may find their way into the circulating tissue of the body and may be demonstrated there, and even in the urine. Bordet and Cuica[2] have reported the presence of bacteriophages in the peritoneal exudate of guinea pigs following three or four intraperitoneal injections of B. coli at intervals of a few days. More recently Pico[3] has reported their presence in pancreatin, trypsin and papain. Combiesco[4] claims to have demonstrated bacteriophages in enterokinase and trypsin. Bachman and Aquino[5] have reported their presence in pancreatin. Kuttner[6] found them present in 25 per cent and 50 per cent glycerol extracts of the small intestines of guinea pigs, and in acetone extracts of normal guinea pig liver. In a recent study I (McKinley)[7] have been unable to demonstrate bacteriophages in various commercial preparations of digestive enzymes and ferments, vitamines, bile salts or in a biologically active preparation of "insulin" which was prepared in this laboratory. Fleming and Allison[8] have reported a lytic "substance" which they find in various secretions and tissues of the body, to which they have given the name "lysozyme." In discussing the relation of this "substance" to the bacteriophage they state that the two are separate and distinct, the greatest difference being that "lysozyme" is not transmissible in series as is the bacteriophage, and the small round clear areas in solid cultures which are characteristic of the bacteriophage do not obtain wth "lysozyme." The experimental work reported below had been begun some time prior to this publication.

Having at our disposal a variety of fresh tissues coming to us each day from the operative clinic for pathologic diagnosis we thought it might be of interest to look for the presence of bacteriophages in these specimens. The specimens are received by us "unfixed" wrapped in oiled paper, for gross descriptions, after which blocks are prepared and sectioned for microscopic diagnosis. No effort was made to control the factor of contamination between the operating table and the laboratory, other than the above. This was a constant factor and had no bearing on our results. When received at the laboratory strict asepsis was practiced. An effort was made in each case to obtain parts of the tissue to be examined which had not been exposed to contamination, by taking fragments from freshly sectioned surfaces

*From the Department of Bacteriology, Hygiene and Preventive Medicine, Baylor University College of Medicine, Dallas, Texas.

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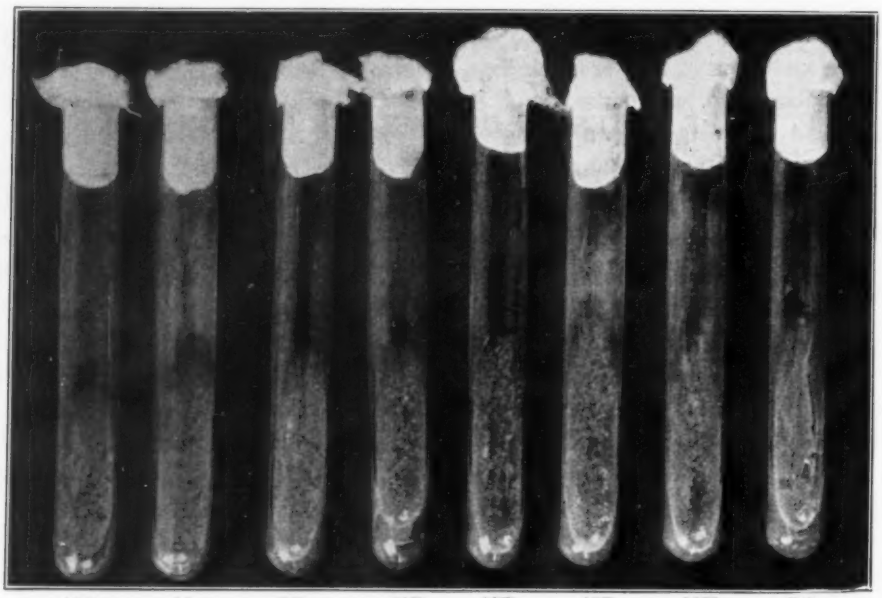

Fig. 1.

42B.—Filtrate from cerumen.
39.—Filtrate from tonsil.
8C.—Filtrate from lymph node.
48B.—48B.—Filtrates from chronic sinus infection.
45B.—Filtrate from prostate gland.
22X.—Filtrate from empyema fluid.
CC.—Control Shiga Dysentery.

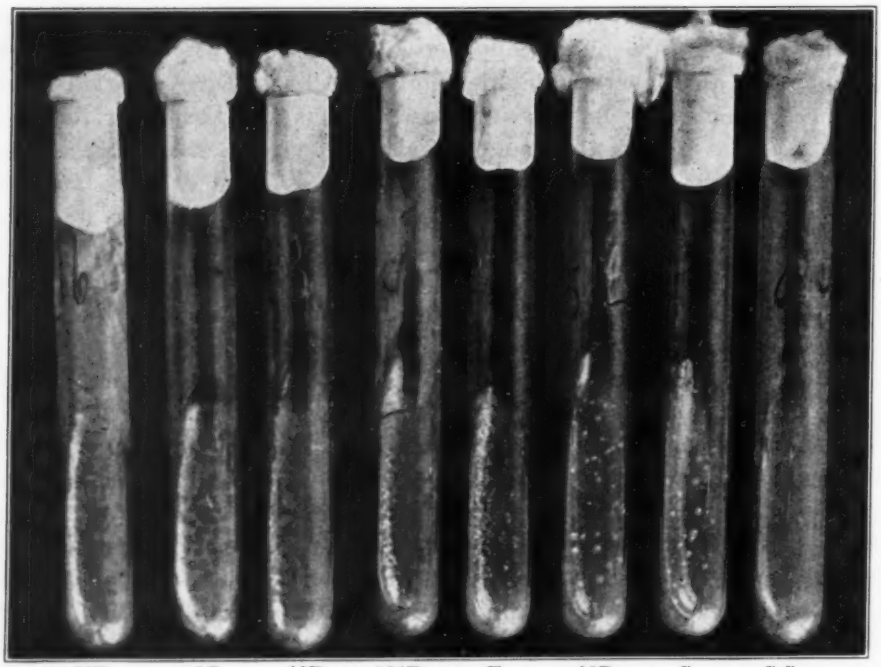

Fig. 2.

16B.—Urine filtrate.
1C.—Lymph gland filtrate.
19B.—Prostatic fluid filtrate.
31B.—Salivary gland filtrate.
E.—GP kidney filtrate.
25B.—Appendix filtrate.
G.—GP lung filtrate.
CC.—Normal Shiga Dysentery.

with sterile instruments. The tissue was cut up into smaller fragments and placed in small flasks containing 50 c.c. of plain broth of pH 8.1. The flasks were then incubated at 37.5° C. for 24 hours, after which the content was filtered through filter paper and then through a mandler No. 4 candle. The filtrates were then incubated for 24 hours to prove their sterility. To test the filtrates, three broth tubes were inoculated with young agar slant cultures of Shiga dysentery (control organism used in testing each filtrate though other organisms were also used), and these broth cultures were incubated from 6 to 12 hours. The filtrates were then tested by adding two, ten and thirty drops of filtrate to the three broth cultures respectively. Suitable broth and culture controls were included in the series. The young broth cultures plus the varied amounts of filtrate and control tubes were then incubated, and readings were taken at 12 hour intervals up to 72 hours. At the end of the first 24 hour period agar slant cultures were prepared from each tube, care being taken to cover the entire surface of the slanted medium with the suspected material. These subcultures were also incubated and inspected at the end of 24 hours for "colonies" of bacteriophages. Plate subcultures were also prepared at the same time, and examined in 24 hours for "sensitive" and "resistant" colonies of the organisms.

In the following tables we have classified the various tissues examined. The pathologic diagnosis has been included as a matter of record, for which I am indebted to Dr. Geo. T. Caldwell.

TABLE I

THE APPENDIX AS A SOURCE OF BACTERIOPHAGES

FILTRATE NUMBER	PATHOLOGIC DIAGNOSIS	COLONIES OF BACTERIOPHAGES	SENSITIVE COLONIES ON PLATES	ORGANISMS WITH WHICH TESTED
7	Acute gangrenopurulent appendicitis	+	+	Shiga dysentery Staph. Albus B. coli
12	Chronic catarrhal appendicitis	–	–	"
13	"	–	–	"
16	"	–	–	"
26	"	+	+	"
27	"	–	–	"
25b	"	+	+	"
23	Acute suppurative appendicitis	–	–	"

All of these filtrates were passed at least through ten series, some of them more. The filtrates were lytic in all cases for at least one of the organisms tested where marked positive, and the lytic principle was easily adapted to the others. One will note that it apparently makes no difference whether the pathologic process is of the acute or chronic type, as two filtrates from chronic appendicitis were positive, and one of two acute types was positive. This seems to us important, in view of the fact that the presence of bacteriophages in the stools of dysentery patients, especially during convalescence, has been emphasized, though d'Herelle has repeatedly stated that they are to be found in normal stools as well.

In neither of these filtrates was there the least suggestion of a lytic

TABLE II

THE PLACENTA AS A SOURCE OF BACTERIOPHAGES

FILTRATE NUMBER	PATHOLOGIC DIAGNOSIS	LYSIS IN BROTH	COLONIES OF BACTERIOPHAGES	SENSITIVE COLONIES ON PLATES	ORGANISMS TESTED
8	Retained Placenta early pregnancy	—	—	—	B. dysentery Shiga Flexner B. coli Staph. Albus Staph. Aureus B. typhosus Para A Para B
17	Retained Placenta early pregnancy	—	—	—	

principle in any of the tests made during ten passages with the different organisms tested.

In working with tonsils the technic, as described above, was modified somewhat. The tonsils were received from the hospital in the same manner described, and portions of each tonsil (being run in pairs) were cut up into fine fragments with sterile scissors, and were then ground with emery in a mortar before placing them in the broth flasks.

TABLE III

THE TONSIL AS A SOURCE OF BACTERIOPHAGES

FILTRATE NUMBER	LYSIS IN BROTH	COLONIES OF BACTERIOPHAGES	SENSITIVE COLONIES ON PLATES	ORGANISMS WITH WHICH TESTED
1	+	+	+	Shiga dysentery Staph. Aureus Staph. Albus
34	—	—	—	"
35	—	—	—	"
38	—	—	—	"
39	+	+	+	"
41	—	—	—	"
44	—	—	—	"
50	—	—	—	"
1a	—	—	—	"
2a	—	—	—	"
3a	—	—	—	"

Most of the ovaries examined contained serous cysts, and before opening these the serous fluid was withdrawn with a sterile needle and syringe. The cystic fluid as well as the ovarian tissue was then tested for bacteriophages. In each case the serous fluid was found to be sterile when examined bacteriologically.

In no instance was there the slightest suggestion of a lytic principle in either the filtrates from the cystic fluid or the ovarian tissue. These filtrates were carried through seven passages before they were discontinued.

Besides the tissues classified above we have examined a variety of other tissues for bacteriophages, which we have included under "miscellaneous tissues" in Table V.

We have, in the course of this study, examined normal and diseased tissues from guinea pigs as well as human tissues. Following the technic outlined by Kuttner we made 50 per cent glycerol extracts of the small intes-

TABLE IV

THE OVARY AS A SOURCE OF BACTERIOPHAGES

FILTRATE NUMBER	PATHOLOGIC DIAGNOSIS	LYSIS IN BROTH	COLONIES OF BACTERIOPHAGES	SENSITIVE COLONIES ON PLATES	ORGANISMS WITH WHICH TESTED
5	Ovarian Cysts	–	–	–	B. dysentery Shiga Flexner B. coli Staph. Albus Staph. Aureus B. typhosus Para A Para B
11	Multiple serous cysts of ovary	–	–	–	"
24	"	–	–	–	"
29b	Slight fibrous periovaritis	–	–	–	"
24b	Multiple serous cysts of ovary	–	–	–	"

TABLE V

MISCELLANEOUS TISSUES EXAMINED FOR BACTERIOPHAGES

FILTRATE NUMBER	TISSUE OR SPECIMEN EXAMINED	PATHOLOGIC DIAGNOSIS	LYSIS IN BROTH	COLONIES OF BACTERIOPHAGES	SENSITIVE COLONIES
3	Fibroid of uterus	Small subserous fibroid	–	–	–
3b	Small intestine of 8 month fetus	Spina Bifida Int. Hydrocephalus. Club feet	+	+	+
4	Mammary gland	Suppurative mastitis	+	+	+
6	Uterus	Medullary carcinoma	–	–	–
19	Fallopian tube	Chronic catarrhal and follicular salpingitis	–	–	–
32b	Mammary gland	Chronic interstitial mastitis	+	+	+
20	Perineum	Chronic suppurative inflammation	–	–	–
21	Tube and Ovary	Chronic tuboovaritis with infected cyst of ovary	–	–	–
22	Polyp	Pedunculated cervical polyp. Chronic cervicitis	–	–	–
1c	Lymph gland	Caseous tuberculosis of cervical lymph node	+	+	+
25	Liver (Autopsy)	Acute yellow atrophy	–	–	–
28	Fecal concretion	Perforated gangrenopurulent appendicitis	–	–	–
33	Gall stone	Moderate fibrosis of gall bladder wall with stones	–	–	–
33x	Gall bladder	As above	+	+	+
36	Lymph gland	Suppurative lymphadenitis of inguinal gland	–	–	–
30b	Mammary gland	Chronic cystic mastitis with early duct cell carcinoma	–	–	–
33b	Thyroid	Adenoma of thyroid	–	–	–
31b	Submaxillary salivary gland	Sialolithiasis with chronic inflammation	+	+	+
39b	Salivary concretion	As above	+	+	+
19b	Fluid from prostatic massage	Normal	+	+	+

These filtrates were tested only with Shiga dysentery and Staph. Aureus.

tine, large intestine, stomach wall and uterus. Acetone extractions of guinea pig liver, kidney, lungs, spleen and heart were made from normal animals and from the liver, lungs, spleen and lymph nodes of guinea pigs dying from experimental tuberculosis, these tissues all being loaded with tubercles. In addition to these we have examined the intestines from normal cats and rabbits.

TABLE VI

TISSUES OF OTHER ANIMALS AND MAN EXAMINED FOR BACTERIOPHAGES

FILTRATE NUMBER	TISSUE EXAMINED	LYSIS IN BROTH	COLONIES OF BACTERIOPHAGES	SENSITIVE COLONIES ON PLATES
A	Normal GP uterus	−	−	−
B	Normal GP sm. Int.	+	+	+
C	Normal GP large intestine	+	+	+
D	Normal GP stomach	+	+	+
E	Normal GP kidney	+	+	+
F	Normal GP heart	−	−	−
G	Normal GP lungs	+	+	+
H	Normal GP liver	+	+	+
I	Normal GP spleen	−	−	−
J	Tuberculous GP Lungs	−	−	−
K	Tuberculous GP lymph nodes	−	−	−
L	Tuberculous GP liver	+	+	+
M	Tuberculous GP spleen	−	−	−
5c	Beef pancreas	+	+	+
9a	Normal rabbit intestine	+	+	+
11a	Normal cat int.	+	+	+
9	Stock bacteriophage for control	+	+	+

All of these filtrates were tested with Shiga dysentery, B. coli and B typhosus and were carried through at least ten passages, some of them more. Those marked positive were either polyvalent for the organisms tested, or were easily adapted.

Stools from fifty-five individuals have been examined for bacteriophages which, for sake of brevity, are not tabulated. Of these fifty-five stools, forty-three were from students or from apparently normal healthy persons. Twelve were from hospital cases none of which were dysentery cases, or showed any gastrointestinal abnormality. Of the total number examined seven were found to contain bacteriophages when tested with Shiga dysentery.

We have attempted to produce bacteriophages by the intraperitoneal injections of B. coli and also with Shiga dysentery into guinea pigs as reported by Bordet and Cuica, but without success. Our animals received from three to eight injections at intervals of three and four days.

TABLE VII

VARIOUS SECRETIONS OF THE HUMAN BODY AS A SOURCE OF BACTERIOPHAGES

FILTRATE NUMBER	SECRETION TESTED	LYSIS IN BROTH	COLONIES OF BACTERIOPHAGES	SENSITIVE COLONIES ON PLATES
49	Urine	–	–	–
22b	Urine	+	+	+
21b	Bile	+	+	+
40b	Saliva	–	–	–
41b	Saliva	–	–	–
42b	Saliva	–	–	–
39b	Smegma (praeputii)	–	–	–
37b	Sebum	–	–	–
42b	Cerumen	+	+	+

These filtrates were tested with Shiga dysentery only.

DISCUSSION

It will be seen that we have examined a large variety of pathologic and normal tissues of both man and animals. Of some thirty-six different sources examined we have found bacteriophages in seventeen. The "substance" is lytic in nature; it is transmissible in series; it is polyvalent in nature in some cases when first isolated, or admits of adaptation when tested for this property; it produces the characteristic areas in solid cultures making possible their enumeration, and produces the abnormal colonies on plate subcultures. We believe this "substance" to be a true bacteriophage.

It is interesting in this connection to recall the work of Smyth[9] who reported the bactericidal influence of tissues from chick embryos to dysentery bacilli and other organisms in 1914-15 which was especially evident in the immediate vicinity of the tissue fragments with a characteristic, clear, bacteria-free halo around the tissue fragment. He was working with growing tissues, however, while we are dealing here with filtrates from tissues. We have wondered what, if any, relation exists between these phenomena. Smyth attributes this antagonistic action of the tissue to "the immediate area of white cell migration," but states further that there is a favorable action to the growth of organisms just beyond this area, which is probably due to cell secretions or excretions diffusing out from the tissue. Kligler[10] also has reported that saline extracts of beef heart, goat blood, rabbit and cat tissues and human secretions influence favorably the growth of a number of pathogenic bacteria studied by him, and believes that the substances involved belong to the class of vitamines. Both Smyth and Kligler, then, believe that the tissue cell secretions or excretions are favorable to bacterial growth and not antagonistic to it. In that event we cannot be dealing with tissue cell secretions or excretions in our filtrates, and the lytic "substance" must be something other than these, or a "substance" obtained under conditions favorable for bacteriophages not obtained when these conditions do not exist. It is interesting to note in what tissues bacteriophages are found, especially the type of tissue. Intestine, mammary gland, gall bladder, salivary gland, prostatic fluid, liver, lung, stomach, lymph gland, tonsil, appendix, pancreas and kidney all show the presence of bacteriophages, while bile and normal stools have also been found to be positive. We have asked our-

selves if the presence of bacteriophages in these tissues could in any way be related to the epithelial structures present in most of them; if secreting epithelium could in any way account for this phenomenon, and more specifically if the zymogenic granulations can in any way be concerned in the process. According to Altmann zymogenic granulations may be bioplasts, independent elements, capable of reproduction by division in a cellular medium. In this connection we recall that of all the filtrates examined, the filtrates from the submaxillary gland and pancreas were the strongest, the most active or virulent of any of the filtrates. The six hour broth cultures became perfectly limpid in the very first series with these filtrates, and it has long been known that the epithelial cells lining the acini of the salivary glands and pancreas are filled, especially when at rest, with fine granules, which are thought to form the specific secretion of these glands. To our mind the finding of bacteriophages in these various tissues presents numerous interesting problems other than those related to origin. The latency of bacteria in tissues of the healthy body is beyond question. Canfora has demonstrated the latency of tetanus spores in tissues of guinea pigs under experimental conditions. The latency of treponema pallidum and many trypanosomes in the circulation and tissues of animals and man is well known. Torrey and others have demonstrated many varieties of diphtheroids from lymph nodes, the seat of various nonbacterial conditions, such as sarcoma and Hodgkins' disease. Rosenau has found diphtheroids and cocci in the blood stream of patients in the presence of febrile conditions, which were not due to the particular organisms isolated. Can this phenomenon of latency of bacteria in tissues be explained by the presence of bacteriophages? What is the relation of bacteriophages in the tissues of man to immunity, and that rather indefinite term "resistance" we speak of in regard to infectious processes? The presence of bacteriophages in tuberculous lymph glands is of extreme interest, in view of the chronicity of this disease. The presence of bacteriophages in the tissues of the gall bladder is, apparently, a constant finding. In addition to the one gall bladder reported in Table V, where bacteriophages were demonstrated to be present, Dr. G. H. Sanders in this laboratory has examined eight other gall bladders all of which were positive. Through his courtesy we submit his results as follows: Filtrates were made from both mucosa and muscularis (the muscularis of six only were examined), and he found that the muscularis was uniformly negative for bacteriophages, while the mucosae were always positive. His filtrates were tested with seven common pathogenic and nonpathogenic organisms and were either polyvalent, or could be adapted to each organism. His mucosa filtrates have been passed through several series with noted increase in virulence of the bacteriophages.

CONCLUSIONS

We have isolated a lytic "substance" for numerous pathogenic organisms from various pathologic and normal tissues of man and animals. This lytic principle is transmissible in series and produces the characteristic clear areas on solid cultures, as do bacteriophages. The lytic principle, furthermore, is polyvalent for many organisms or admits of adaptation. We believe it to

be a true bacteriophage, since it possesses the essential characteristics of bacteriophages and not a separate and distinct substance such as "lysozyme." We feel that these experiments are very suggestive as to the diastatic nature of bacteriophages: that the "substance" in the tissue cell, or produced by the tissue cell, initiates the lytic phenomenon observed with young generations of various bacteria, following which the lytic principle is augmented quantitatively and thereby in concentration by a similar "substance" from the lysed bacterium. This would explain the increase in "virulence" of the bacteriophage and also its transmissibility in series, as the lytic "substance" would ever be increasing in concentration when exposed to a bacterial cellular medium. This much seems certain, that there is "something" within tissue cells, whether epithelial, lymphoid, leucocytic, lymphocytic or what not in type, which acts as, or is the origin of, the lytic principle known to us as the bacteriophage; a "substance" which is capable of "reproduction," or "multiplication," in a cellular medium such as bacterial suspensions, a "multiplication" which takes place at the expense of the bacterial cells.

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ISOAMYL ETHYL BARBITURIC ACID—AN ANESTHETIC WITHOUT INFLUENCE ON BLOOD SUGAR REGULATION*

By Irvine H. Page, Indianapolis, Ind.

INVESTIGATIONS involving the mechanism of blood sugar regulation as studied in animals have been badly handicapped for the lack of a suitable anesthetic that does not in itself markedly influence the sugar of the blood. Anesthetics suitable for such work must, first, produce surgical anesthesia; second, involve no appreciable interference with normal blood sugar regulation; third, have a low degree of toxicity, and finally, give an even degree of anesthesia requiring slight attention from the operator. It is believed that such an anesthetic is found in isoamyl ethyl barbituric acid. The following experiments will illustrate its value and compare it with certain closely allied substances.

EXPERIMENTAL WORK

Isoamyl ethyl barbituric acid is comparatively insoluble in water. A solution is best made by adding 8.85 c.c. N/2 NaOH per gram, then heating to the boiling point. Excessive heating should be avoided since it is found that hydrolysis takes place, thus reducing its anesthetizing power.

Administration by stomach tube has been found to give more desirable results than subcutaneous injection, especially if it be desired that the animal survive operative procedures.

Animals that have been starved (24 hours in the case of dogs, and 48 hours in rabbits) are better adapted to this form of anesthesia than those that have been well fed. In order to insure a rich glycogen supply, sucrose administration does not appear objectionable.

DOSAGE

Doses of 0.10 to 0.125 grams per kilo. by stomach or 0.09 to 0.11 grams subcutaneously in dogs produces surgical anesthesia in 1 to 3 hours lasting

Table I

Degree of Anesthesia Induced by Isoamyl Ethyl Barbituric Acid

DOSAGE PER KILO	WT. RABBIT	ANESTHESIA	TIME OF ONSET IN MINUTES	AFTER 5 HOURS
.017	2680	*	30	S
.04	1250	*	25	S
.05	1795	*	30	S
.06	1920	**	30	S
.07	1550	***	35	S
.10	1520	***	35	S
.12	2075	****	25	D
.15	2310	****	25	D

*—Drowsy, **—Barely can be roused, ***—Cannot be roused, ****—Very deep anesthesia, S—Survived, D—Dead.

*From the Eli Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.
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18 to 24 hours. Rabbits generally require 0.08 to 0.10 grams per kilo. by stomach and 0.06 to 0.08 subcutaneously. In the latter animal the anesthesia comes on rapidly, 5 to 15 minutes, but lasts only 3 to 6 hours.

Light anesthesia suitable for the isolation of the carotid artery, etc., requires a dosage of 0.04 to 0.06 grams per kilo. by stomach or subcutaneously. Table I illustrates the relation between dosage and degree of anesthesia.

EFFECT ON BLOOD SUGAR

Blood sugar was determined by the Schaffer-Hartmann[1] method. Table II is an example taken from our protocols of forty rabbits. The variations that occur are well within physiologic limits.

TABLE II

ISOAMYL ETHYL BARBITURIC ACID EFFECTS ON BLOOD SUGAR OF NORMAL RABBITS

WT.	DOSAGE PER KILO	BLOOD SUGAR							
		BEFORE	AFTER MINUTES 15	30	45	1½ HRS.	3½ HRS.	6 HR.	7½ HR.
1320	.04	.0975	.0975	——	.0975	——	——	——	——
987	.06	——	——	——	——	.0812	.0912	——	.1000
1837	.06	.1025	——	——	——	.1062	.1000	.0975	——
1855	.06	.1000	——	——	——	.1062	.0975	.1000	——
1020	.08	.0975	——	——	——	.0975	.1025	——	——
1270	.10	.1100	.1100	——	.1100	.1025	——	——	——
3720	.10	.1000	——	.1000	——	.1000	.1000	.1065	——

SPLANCHNIC STIMULATION

Rabbits under the influence of isoamyl ethyl barbituric acid show normal responses to splanchnic stimulation. In one case the splanchnic nerves were isolated by incision through the posterior route. The isolated nerves were stimulated electrically 4 seconds. The blood sugar results were as follows: Normal –0.0937; 3 minutes after stimulation –0.1025; 10 minutes after –0.1100; 35 minutes –0.1493. Similar results have been obtained with dogs.

Likewise it has been found that with this anesthetic the usual hyperglycemia following hemorrhage occurs.

TABLE III

INSULIN HYPOGLYCEMIA

TIMES AFTER ANESTHETIC ADMINISTRATION	INSULIN INTRAVENOUSLY	BLOOD SUGAR	NOTES
3 hours	.....	.....	Femoral vein and artery dissected out.
5 "	.....	.1325	
5 "	5 units	.....	
5 " 15 min.		.0812	
5 " 30 "		.0718	
6 " 30 "		.0718	
10 "		.0850	
10 " 15 "	5 units	.....	
10 " 30 "		.0425	
10 " 45 "		.0325	
11 "		.0200	
11 " 45 "		.0520	

Dog weight, 5.45 kg. 0.125 grams isoamyl ethyl barbituric acid per kilo.

INSULIN (ILETIN, LILLY)

In order to demonstrate that the organism not only reacts to produce typical hyperglycemias, but also hypoglycemias, while under the influence of isoamyl ethyl barbituric acid, insulin was used.

By plotting blood sugar against time it will be found that the area covered by the two curves is practically equal. It is believed that the nervous influence in glycogenolysis has been minimized by the anesthesia. This is, of course, to be desired in the potency testing of blood sugar regulators such as insulin.

SIMILAR ANESTHETICS

A few anesthetics of similar nature have been investigated simply for comparative purposes. Without going into detail it may be said that none of the substances—barbital, isopropyl ethyl barbituric acid, urethane and trichlorotertiary butyl alcohol—maintained as uniform a level of blood sugar as the isoamyl ethyl barbituric acid. Shonle and Moment[2] have shown that the toxicity of the latter is lower, wth greater anesthetizing power, than any of the above. Tatum and Parsons[3] have shown that barbital is a good anesthetic for certain purposes, but for blood sugar work isoamyl ethyl barbituric acid appears superior. Like barbital, isoamyl ethyl barbituric acid does not affect blood pressure.

CONCLUSIONS

1. Isoamyl ethyl barbituric acid has been shown to have little or no influence on blood sugar in amounts sufficient to produce surgical anesthesia.

2. Under this anesthesia splanchnic stimulation and hemorrhage induce typical hyperglycemias.

3. Insulin produces a uniform decrease in blood sugar in animals under this anesthetic, probably due to the fact that the nervous factor in glycogenolysis is minimized.

4. Isoamyl ethyl barbituric acid has been found superior to barbital, isopropyl ethyl barbituric acid, trichlorotertiary butyl alcohol and urethane for blood sugar investigations.

The author wishes to express his sincere appreciation to Dr. G. H. A. Clowes, Mr. Edward Swanson, Mr. H. Shonle, Mr. W. O. Roeder and Dr. A. L. Walters for their interest in this investigation.

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LABORATORY METHODS

AN ARTIFICIAL RESPIRATION AND ETHER APPARATUS FOR USE WITH COMPRESSED AIR*

By H. F. Pierce, A.B., B.Sc., (Oxon), New York, N. Y.

WHEN compressed air is employed for the administration of artificial respiration the flow of air must be interrupted a certain number of times per minute in order to simulate breathing. This is usually accomplished by means of automatic valves such as have been described by W. T. Porter, W. B. Cannon, and others. These devices, while satisfactory for research, present certain disadvantages when numbers are required for

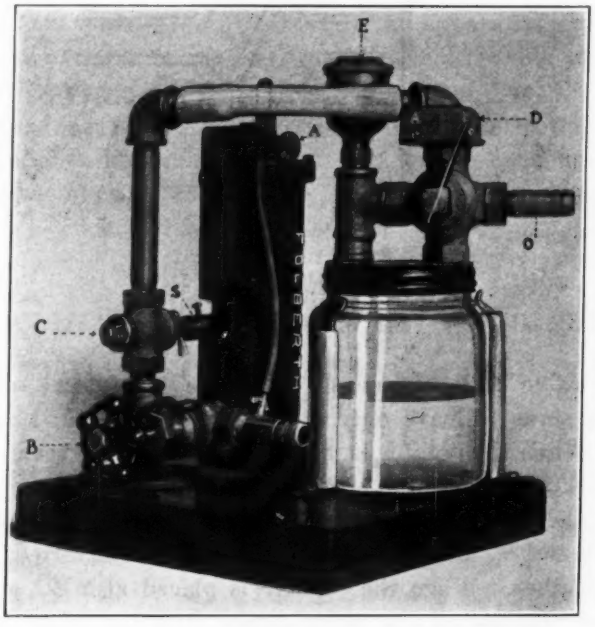

Fig. 1.

student courses. For example, the electric motor driven interrupter is rather expensive, and the tip-bucket valve requires running water to operate it and a sink to carry away the waste.

In the apparatus here described, power for operating the interrupter valve is derived from the compressed air, acting through the agency of a reciprocating air motor similar to those used in automobile windshield wipers.

*From the Department of Physiology, Columbia University, New York City.
Received for publication, August 14, 1923.

Referring to the accompanying photograph, (Fig. 1), compressed air from the mains enters the apparatus at H. A part of the stream is shunted through the small vertical tube to the motor, which is seen in the background at the left. Another part passes through the needle valve B, the interrupter cock C, and the ether apparatus, to the animal.

The speed of the motor, and hence the number of respirations per minute, is controlled by the small knurled screw A. The amount of air passing to the animal is regulated by the needle valve B. By loosening the set screw S and rotating the sleeve on the motor shaft, the period of the inspiratory phase may be lengthened or shortened. By stopping the motor during the inspiratory phase of the cycle a steady stream of air may be obtained for tracheal insufflation.

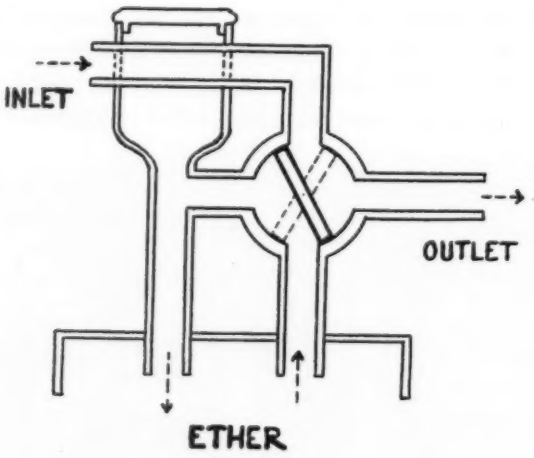

Fig. 2.

The ether valve (Fig. 2) consists of a 4-way steamcock, altered by filing away the plug so as to leave only a thin ward of metal, 1 millimeter thick at the edges. When the pointer is placed at "A" on the scale D, air from the artificial respiration apparatus passes in at the top of the valve and out through O by the most direct route. In this position both openings into the ether bottle are tightly closed, and the bottle may be inverted without danger of spilling the ether. When the pointer is placed at "E" on the scale, the air entering at the top of the valve passes into the ether bottle, over the surface of the ether, back through the valve, and out at O. By placing the pointer at intermediate positions between "A" and "E", various mixtures of air and ether vapor may be obtained. The drawing shows diagrammatically the path taken by the air in passing through the ether valve. The plug is shown in heavy lines in the position it occupies when the pointer is at "A" on the scale. The light lines show its position when the pointer is at "E".

In Fig. 1 it will be noted that the ether valve is connected to the respiration apparatus by means of a short rubber tube. This tube may be

easily removed when artificial respiration is not required and the ether bottle then used in the ordinary way. The bottle may be replenished with ether while the apparatus is in use by removing the milled cap E.

A word about the motor* may be of interest to laboratory workers. It consists of a cylindrical casing in which two pistons reciprocate under control of mechanically actuated valves. Power is transmitted by means of a rack and pinion to the shaft, which rotates through an angle of approximately 120 degrees and then reverses. There are a number of air motors on the market, and the one used in this apparatus was selected because of its excellent mechanical features and sound construction. Two types are available, operating respectively on compressed air and on suction. The first type is used in the apparatus here described. It works satisfactorily on pressures as low as 2 pounds per square inch.

The suction type of motor operates on the vacuum obtained from an ordinary water aspirator, and may be used as a source of power for rocking photographic trays, operating gas analysis apparatus, shaking Clark H ion electrodes, etc.

In this laboratory air is supplied to the machines under a pressure of 5 pounds per square inch. In an emergency the apparatus may be connected through a reduction valve to an oxygen cylinder, in which case oxygen and artificial respiration may be administered at the same time.

Eight of these machines have been in constant use in the hands of first year medical students during a period of four months, and no repairs or adjustments have yet been necessary.

THE KOLMER QUANTITATIVE TEST FOR SYPHILIS†

COMPARISON WITH A TECHNIC USING AN ANTI-HEN HEMOLYTIC SYSTEM

By Carl W. Maynard, M.D., Pueblo Clinic, Pueblo, Colorado

THIS report compares the Kolmer quantitative technic[1] with a method using an anti-hen hemolytic system. The Kolmer test for syphilis has received wide recognition among clinical pathologists as a candidate for the "standard" technic. It is being compared with many technics, in many laboratories. The published reports are largely favorable to the new method, as yielding more sensitive reactions than those formerly in use.

The writer has used hen red cells in his serologic work for the past nine years, and has found that only a negligible number of human sera contain natural anti-hen amboceptor. For this reason it might be expected that, all

*Manufactured by the Folberth Auto Specialty Co., Cleveland, Ohio. The complete apparatus, as described, is supplied by Joseph Becker, 437 West 59th Street, New York City.

†Received for publication, August 24, 1923.

other factors of the two tests being controlled, the one using an anti-hen hemolytic system would give positive reactions on some bloods which are negative with an anti-sheep system. This would be true, of course, only if no attempt were made to remove the natural hemolysins.

The present report covers a total of 806 tests made with both my old technic, using hen erythrocytes, and the Kolmer quantitative method, using sheep cells. The data include 752 blood sera and 54 spinal fluids. Five hundred and forty-nine blood sera and 29 spinal fluids were negative with both technics. Two hundred and three sera and 25 spinal fluids produced more or less complement fixation with the older method. The results are grouped in Table I to show the comparative strength of the reactions with the old technic and with the method of Kolmer.

TECHNIC

The method used in this laboratory has been developed gradually over ten years. The anti-hen hemolytic system has survived changes in other details.

Hemolytic System.—Red cells; hen, 1 c.c. of 2 per cent emulsion.

Amboceptor; in dilution so that two units are contained in approximately 0.1 c.c. of the diluted serum, the exact amount being determined by titration just prior to each test. Dose=two units.

Complement; 40 per cent dilution of fresh mixed serum from three or more guinea pigs, titrated before each test with two units of amboceptor. Two units are used in the test.

Antigens.—(1) Cholesterinized heart extract (human or beef). (2) Syphilitic liver extract. (3) Acetone insoluble lipoids.

Serum.—0.1 c.c. of serum, heated for 30 minutes (recently 15 minutes) at 56° C., with antigens 1 and 2.

0.025 c.c. serum, unheated, with antigen 3.

Spinal fluid is used in 0.2 c.c. and 1.0 c.c. amounts, with all antigens, and is not heated.

Controls.—Known positive and known negative sera, with the usual antigen and serum controls.

Total Quantity.—Made up to 2 c.c. with 0.85 per cent sodium chloride.

Fixation of Complement.—One hour in the ice water-bath (6-8° C.), followed by one hour in the warm water-bath at 37° C. This has been found to give more clear-cut reactions, without increasing the number of faint positives over those obtained by only the single incubation at 37° C.

Following the first fixation period, the amboceptor is mixed with the red-cell emulsion in such amount that 1 c.c. contains two units, and the mixture is heated at 37° C. for one hour. It is then added to the previously incubated tubes, and these replaced in the water bath for one hour, at the end of which time preliminary readings are made. The final readings are made after 14 hours in the ice-box.

In *recording findings,* the degree of fixation in each antigen-containing tube in order is expressed as minus, one, two three, or four plus; i.e. 444

would mean complete fixation with all three antigens; 4—, complete fixation with the cholesterinized antigen and no fixation with the other two; and so forth.

KOLMER TECHNIC

The Kolmer quantitative technic has been followed exactly as described in the *American Journal of Syphilis,* with one exception. The heating of complement with antigen at 37° C. for one hour before titrating was found to weaken the complement so that later titration gave 1:30 as an average dilution to be used in 1 c.c. dose for the final test. This was a decided overdose. One hour in the ice water-bath seemed to approximate more nearly the actual condition as developed during the ice-box fixation in the test itself. This change was made, and yields a complement unit of proper strength; at least such a unit as would not encourage false negative reactions.

Kolmer's later serum dilutions, i.e. 0.1, 0.05, 0.025, 0.005, and 0.0025 c.c.. have been used throughout.

RESULTS

A study of Table I shows that there is a general agreement in 160 (70 per cent) of the 228 tests that were positive with my technic. Sixty-eight (30 per cent) of the 228 give no fixation whatever with the new Kolmer method.

In no instance has the Kolmer technic given a positive reaction and the old technic a negative.

The discrepancies between the two technics are analyzed in Table II. A study of this table will show that:

1. The weaker reactions with my test are most frequently joined with a negative Kolmer.
2. Twenty cases of treated syphilis, and five untreated cases, are included.
3. Eleven cases are clinically not luetic.
4. The other 32 cases are obscure brain, eye, ear, nerve and pelvic conditions, which may or may not have been due to, or accompanied by, latent syphilis.

DISCUSSION

This comparison was begun with the definite hope that the Kolmer test, as performed in this laboratory, might be found worthy of adoption to supersede the old method. Kolmer has furnished the antigens for both technics, and has aided kindly with counsel.

My rule has always been to urge conservatism in the interpretation of results. No condition is diagnosed as syphilis from a single positive Wassermann, except in the presence of definite clinical evidence of syphilis. Any result less than a 444, in diagnosis, is supposed to be checked by repeated tests. The two- and the one-plus reactions are considered as essentially negative, except in treated cases.

Keeping the above in mind, my early impression was that the new technic

was giving negative results only in cases in which the positive with the anti-hen method might be suspected of falsity. After summarizing the entire series, however, it seems that the 11 false positives (Table II) are more than balanced by the 25 cases of proven lues, which might have been encouraged to neglect treatment had they been advised only by the negative findings of the Kolmer test.

TABLE I

NO. OF TESTS	RESULTS WITH OLD METHOD	KOLMER MORE OR LESS FIXATION IN FIRST					
		5 TUBES	4 TUBES	3 TUBES	2 TUBES	1 TUBE	NONE
63	444	32	12	14	3	0	2
54	44?	16	7	24	3	0	4
19	4??	1	2	10	1	0	5
23	333	0	0	3	1	0	19
44	Mixed 1, 2, and 3	0		6	1	1	35
Fluids			2				
15	444	4		6	1	2	0
3	4??	0	0	1	2	0	0
7	Mixed 1, 2, and 3	0	0	1	1	2	3
228		53	24	65	13	5	68

TABLE II

OLD METHOD	KOLMER	SYPHILIS UNTREATED	SYPHILIS TREATED	BRAIN TUMOR	EYE, EAR, SPINAL CORD, PELVIS	NOT SYPHILIS
444	.....		1		1	
44?	.....	1	2	1		
4??	.....		3		2	
333	.....		7	1	6	5
Mixed 1, 2, and 3	.....	2	7		21	5
Fluids						
Mixed 1, 2, and 3	.....	2				1
		5	20	2	30	11

A particularly interesting observation of the comparative sensitiveness of the two tests was shown in a case in which the Treponema pallidum was demonstrated by dark-field in serum from a primary sore. Blood taken from this patient ten days after the appearance of the ulcer gave 222 with the old technic, and was negative with the Kolmer. On the fourteenth day the old method gave 333, and the Kolmer -22- -.

SUMMARY

Of 228 cases giving more or less complement fixation with an anti-hen hemolytic system technic, 68 (30 per cent) were completely negative with the Kolmer quantitative test. Of these 68 cases, 25 were known syphilitics, 32 were possibly syphilitic, and 11 were not syphilitic.

The use of an anti-hen hemolytic system in a modified combination of Wassermann and Noguchi methods yields a high percentage of positive reactions, 5 per cent of which (weakly or moderately positive) are false. No reactions in which one or more antigens give complete fixation have proved clinically false. False weak positives, from a technic whose results are conservatively interpreted, need give no trouble.

CONCLUSIONS

The Kolmer test for syphilis gives, in this series, false negative findings in 11 per cent of cases positive with an older method. No false positives by the Kolmer method have been demonstrated.

The false negatives can be avoided by using an anti-hen hemolytic system, with several antigens. This technic gives 5 per cent of weak positives on patients who are clinically negative. The frequency of these false positives is more than balanced by the failure of the Kolmer technic to give a positive reaction in 11 per cent of cases with known syphilis.

REFERENCE

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A QUANTITATIVE METHOD FOR THE DETERMINATION OF TOTAL PHOSPHORUS IN THE BLOOD*

By C. S. Smith, Ph.D., M.D., and A. L. Brown, A.B., Columbus, Ohio

INTRODUCTION

THE methods for the estimation of phosphorus in the blood found in the literature of today are based upon colorimetric[1, 2] or nephelometric procedure.[3] The method here described is different, in at least two respects, from any of those published. These differences are, first, a different way of preparing the sample, and second, the volumetric method of determining the phosphorus.

PRINCIPLE

The method is based upon getting the phosphorus into solution as phosphoric acid, and then separating it from other constituents in the blood. A known amount of whole blood is treated with a small amount of magnesium nitrate. The mixture is then carefully heated to dryness. After cooling, the residue is taken up in water and hydrochloric acid and again heated. Nitric acid is added, followed by the addition of ammonium hydroxide and then an excess of nitric acid. Ammonium molybdate is next added and the phosphorus precipitated as the well known "yellow precipitate." The solution is filtered and the precipitate thoroughly washed with a dilute solution of potassium nitrate.

The "yellow precipitate" is dissolved in N/10 sodium hydroxide and the excess alkali titrated by means of N/10 nitric acid.

SOLUTIONS AND REAGENTS

1. Magnesium Nitrate.—Approximately 16 grams of calcined magnesia are dissolved (after adding a small amount of water) in 50 c.c. of concentrated

*From the Laboratory of Physiological Chemistry, College of Medicine, Ohio State University.

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nitric acid. An excess of magnesia is then added and after heating for a few minutes, the solution is filtered and diluted to 100 c.c. with water.

2. Molybdate solution.—Dissolve 25 grams of molybdic acid in 100 c.c. of ammonium hydroxide (sp. gravity 0.96). This solution is cooled and poured in small portions at a time with shaking into 300 c.c. of dilute nitric acid (sp. gravity 1.20). The container holding the acid should be cooled by cold water during the mixing. After mixing, the solution is diluted to 500 c.c. One c.c. of the solution will precipitate 0.001 gram of phosphorus.

3. Standard Sodium Hydroxide (N/10).—One-tenth normal solution is best adapted for this method. Based on the following reaction 1 c.c. of such a solution is equivalent to 0.135 milligrams of phosphorus:[4]

$$12MoO_3(NH_4)_3\ PO_4 + 23\ NaOH = 11\ Na_2\ MoO_4 + (NH_4)_2\ MoO_4 + Na(NH_4)HPO_4 + 11\ H_2O.$$

The standard alkali is prepared in the usual manner by dissolving 4.00 grams of sodium hydroxide in water and diluting to 1000 c.c. The solution should then be titrated against N/10 acid.

4. Standard Nitric Acid.—This is made so that 1.0 c.c. of it will exactly neutralize 1.0 of the N/10 NaOH using phenolphthalein as the indicator. The approximate standard may be prepared by diluting 7.0 c.c. of concentrated nitric acid to 1000 c.c. with distilled water.

5. Potassium Nitrate.—A 1 per cent solution is required.

METHOD

Two cubic centimeters of whole blood (oxalated) are measured into a small porcelain evaporating dish. With stirring, add 10 c.c. of magnesium nitrate solution. Heat is then slowly applied and the mixture stirred at frequent intervals (a very small flame should be used to prevent sputtering. A micro-burner is suggested). The heat is gradually increased and the mass evaporated to dryness. Apply stronger heat to burn off any carbon particles that may be present. The residue at this point must be white or light straw-colored. Allow the dish and contents to cool. After cooling, add 10 to 15 c.c. of water and 5 c.c. of concentrated hydrochloric acid. Heat and stir the mixture. No carbon particles should be present and the solution should be clear thus making filtration unnecessary. The solution is now washed into a beaker with water and an excess of concentrated nitric acid added. This is followed by the addition of a large excess of ammonium hydroxide in order to form ammonium nitrate. Now add concentrated nitric acid again until barely acid, and follow with the addition of about 25 c.c. of ammonium molybdate solution with constant stirring. (It is advisable to add the molybdate solution slowly and with constant stirring in order to form a precipitate that will settle quickly). Allow the solution to stand for at least one hour and then filter through a 11 cm. close-grained quantitative filter paper. Thoroughly wash the residue with a dilute solution of potassium nitrate in order to remove the nitric acid. After washing, place the filter paper and contents in a beaker and add 30 to 40 c.c. of water and 30 c.c. of N/10 sodium hydroxide to dissolve the yellow precipitate. The solution is then

stirred and several drops of phenolphthalein indicator added. The excess of NaOH is titrated with N/10 nitric acid. From the number of cubic centimeters of sodium hydroxide used, the amount of phosphorus is calculated (1.0 c.c. of N/10 NaOH is equivalent to 0.135 milligrams of phosphorus).

EXPERIMENTAL DATA

Several solutions have been prepared containing a known amount of a phosphorus compound and the amount put into the solution was practically recovered by this method. The error in all cases was less than 1.0 per cent.

KNOWN P IN SOLUTION MG. PER 100 CC.	P OBTAINED BY THIS METHOD	PERCENTAGE ERROR
56.5	56.0	0.9%
56.5	56.8	0.5%
56.5	56.6	0.2%
65.2	65.5	0.2%
65.2	65.0	0.1%
65.2	65.7	0.8%

On several samples of normal human blood the phosphorus content was found to be 55 to 60 milligrams per 100 c.c. of blood.

COMMENTS

1. There must be an excess of ammonium nitrate present in order to completely precipitate the phosphorus.

2. It is of the utmost importance to wash the yellow precipitate with KNO_3 until free of acid as the slightest trace of acid would ruin the titration.

3. Stirring, while adding the ammonium molybdate solution, is necessary in order to obtain the precipitate in proper form for rapid filtration.

4. Precipitation of the yellow precipitate takes place more rapidly in warm than in cold solutions. The temperature should not exceed 65° C. during precipitation as a higher temperature tends to separate molybdic acid from the solution.

5. A complete determination can be made in about three hours.

SUMMARY

1. A method is described for the determination of total phosphorus in the blood, based upon titrating the well known yellow precipitate with 0.1 N NaOH.

2. The method here described differs from others in the procedure followed to convert the phosphorus in the blood into phosphoric acid.

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A CONVENIENT METHOD OF DETERMINING PHENOLSULPHONEPHTHALEIN OUTPUT IN THE URINE*

By Vera E. Rothberg, A.B., Pittsburgh, Pa.

IN accordance with the technic originally outlined by Rountree and Geraghty[1] for determining the amount of phenolsulphonephthalein eliminated in the urine after injection of the dye, the urine, rendered alkaline by the addition of sodium hydroxide, is diluted to 500 or 1000 c.c. and read against a known standard in a suitable colorimeter.

The Hellige colorimeter, as modified by the authors of the method, is perhaps the most convenient type of instrument for this purpose, as the percentage of phthalein excreted can be read off directly from the vernier. With a plunger type of colorimeter a simple method has been found of use in this laboratory. After the specimen has been made alkaline, it is diluted, if necessary, until it approximately matches the color of the standard, (1 c.c. of phenolsuphonephthalein solution in a liter). After the final volume of the unknown has been noted, the comparison is made, the cup containing the standard solution being set at 10 mm. on the colorimeter scale. Under these conditions, the final result may be calculated by the use of the formula: $\frac{\text{amount of final dilution of urine (D)}}{\text{reading of unknown (R)}} =$ per cent phenolsulphonephthalein.

This formula was derived in the following way:

The standard, which represents 100 per cent, contains in 1000 c.c. the same amount of dye as has been injected into the patient. If the unknown has been diluted to match the standard exactly, then the reading of the unknown is equal to the height at which the standard has been set. Under these circumstances, the final dilution of the urine specimen, being represented by D, the specimen contains $\frac{D}{1000}$ of 100 per cent or $\frac{D}{10}$ per cent phenolsulphonephthalein.

Since, however, the unknown rarely matches the standard perfectly, the colorimetric reading of the specimen must be taken into consideration, and the factor $\frac{D}{10}$ per cent must be multiplied by the fraction $\frac{\text{height of standard}}{\text{reading of unknown}}$.

If S represents the height at which the standard has been set and R stands for the reading of the unknown, the formula becomes

$$\frac{S}{R} \times \frac{D}{10} = \text{per cent phenolsulphonephthalein.}$$

*From the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.

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With the standard set at 10 and this value substituted for S in the formula above, the equation reads

$$\frac{10}{R} \times \frac{D}{10} = \text{per cent phenolsulphonephthalein.}$$

Simplifying this, we have

$$\frac{D}{R} = \text{per cent phenolsulphonephthalein,}$$

which is the formula given, or, the dilution of the unknown divided by the colorimetric reading of the unknown gives the per cent phenolsulphonephthalein in the urine specimen being examined.

When the alkalinized specimen is paler in color than the standard, before any water has been added, the comparison is made with the undiluted urine, the total volume of the urine and hydrate being substituted for D in the formula.

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EDITORIALS

Dreyer's Antigens

IN May of this year a publication by Prof. Georges Dreyer, of Oxford, appeared, concerning new principles in bacterial immunity.

Dreyer argues that, in general, better immune responses are found after inoculation of Gram-negative and nonacid-fast bacteria than can be determined after inoculation with Gram-positive and acid-fast organisms. The conditions of acid-fastness and Gram-positiveness are associated with the presence in the bacterium of certain waxy, fatty or lipoid substances. These are generally referred to as "lipoidal substances." Such substances protect the specific bacterial proteins and so enable the bacteria to resist tryptic digestion and prevent, following inoculation, the "liberation from the body of the bacterium of the sole adequate stimulus for the immunity reaction of the infected organism."

The tubercle bacillus having a highly developed lipoid constituent was first selected for experiment, and it is the published results of these experiments which will be discussed.

While treatment of tubercle bacilli with formalin alone did not render them nonacid-fast, subsequent extraction with acetone succeeded in doing

so. Gram-positive microorganisms treated in a similar way became Gram-negative. The subsequent inoculation of antigens prepared in this manner resulted in the production of immune bodies, precipitins and agglutinins, in experimental animals. Gram-negative bacteria treated in the same way did not lose their antigenic qualities.

Incomplete reports are given on the treatment of four tuberculous guinea pigs and three tuberculous rabbits. The treatment of the guinea pigs with the "defatted antigen" started at the end of December 1922, that of the rabbits the middle of February 1923. The report is published in May. The tuberculous disease in these few guinea pigs was brought about by the inoculation some months previously of a culture of slight virulence. The virulence of the bovine culture used to infect the rabbits, was sufficient to kill a control in about four months. Evidence regarding the improvement of these infected animals is largely claimed on the increase in weight, the decrease in size of palpable lymph nodes and spleen, and the partial healing of the original sinus at seat of infection. Only in one case is an autopsy reported, and that is of a guinea pig which died suddenly after having young.

While it is perfectly possible that subsequent and more thorough research may confirm these results of Dreyer, yet to those who have done experimental work in tuberculosis the results so far published are far from convincing, and most incomplete and hurried. Guinea pigs infected with attenuated tubercle bacilli can give evidence of all shades of infection, and of recovery, even without treatment. In all probability immune bodies, agglutinins and precipitins, while playing important rôles in some immunity processes, play little part in tuberculosis immunity. Tuberculosis would appear to be a disease in which tissue reaction is paramount.

The inoculations of the "defatted" antigen do not result in reaction response, yet it has been to "reaction" that previous investigators have considered the good and bad effects of tuberculin to be due. It would seem that Dreyer may have a tuberculin of low reacting capacity, but as pointed out by A. K. Krause in 1910 the addition of lipoid substance makes an antigen much more potent. "Defatted" antigens are not strictly new in tuberculosis experimentation. We have seen such advocated by Deycke, Much, studied by Fornet and Wassermann and in this country the "watery" extract of Von-Ruck had extensive trial.

The reports added of treatment of human tuberculosis with the "defatted" antigen state that no local, focal or general reaction followed. In due time fuller reports are promised. The treatment of only four cases of pulmonary tuberculosis is referred to by Drs. Fildes and Western of the London Hospital.

The world has been greatly excited by Dreyer's publications, and yet, to us, it seems sad indeed that the unfortunate victims of tuberculosis should have been rendered too hopeful of a cure on such incomplete evidence.

—G. B. W.

Jaundice

ALTHOUGH much has been accomplished in the study of icterus, and many theories exist regarding its production in the different diseases, successful differentiation of the various types of jaundice has, perforce, awaited the development of satisfactory laboratory reactions. Past attention has been concentrated chiefly upon the presence or absence of the bile pigments and salts in the intestinal contents and in the urine. Increasing study of biliary pigments in the circulating blood with the development of quantitative reactions have developed a clearer insight into the modes of production of jaundice in different diseases.

Many methods for the detection of bile in the blood serum have been described, such as those of Fauchet, Haselherst, etc., but probably the most successful has been that proposed and used by van den Bergh. Van den Bergh has recognized two distinct forms of bilirubin in the serum or plasma of icteric individuals. A modified diazo reagent is used. One form of bilirubin reacts immediately on contact with the development of an intense purplish color, while the reaction with the second variety occurs only after an interval of many minutes. A study of these two varieties of bilirubin has enabled further differentiation of types of jaundice.

Virchow conceived the possibility that jaundice might be either hepatogenous or anahepatogenous. Following the work of Minkowski and Naunyn, it was quite generally understood that all jaundice was hepatogenous in origin. Eppinger in 1908 even took the stand that all forms of jaundice were essentially obstructive, from obstruction somewhere between the larger bile ducts and the smallest bile capillaries. By 1912 this view had come into question, and now we have in rough measure returned to the original Virchowian principle.

Van den Bergh found an immediate serologic reaction in obstructive jaundice, while in hemolytic icterus and in hemorrhagic effusions in the chest or abdomen the reaction was of the delayed type. In other words, with obstructive jaundice a different kind of bilirubin appears in the blood from that found in jaundice resulting from blood hemolysis. When bilirubin has passed through the hepatic parenchymal cells into the bile capillaries it has been changed to a slightly different form from that which is still circulating in the blood. If, because of obstruction lower down, it is resorbed into the circulation, its presence is made manifest by an immediate reaction in van den Bergh's test. That the change in the chemical constituency of the bilirubin occurs in the liver cells is indicated by the observation that gall bladder bile always gives an immediate or typical prompt reaction, while that from hemorrhagic effusions gives a delayed reaction.

The reaction may be biphasic, with both prompt and delayed reactions indicating the simultaneous presence of both types of bilirubin.

Although the polygonal cells of the liver probably rarely escape some damage in obstructive jaundice, those cases in which the major pathology consists of parenchymatous damage are usually better classified under toxic and infectious jaundice. This latter occurs as a complication of many acute

fevers, after the administration of certain drugs, in acute yellow atrophy and in chronic passive congestion. Here, the frequent presence of biphasic reactions indicates that the jaundice may be due in part to parenchymatous damage preventing the passage of bilirubin through the cells, and in part to obstruction in the finer bile capillaries, forcing some of the altered pigment back into the blood stream. Some fails to pass the diseased liver cells, while some of the remainder is transferred through other less seriously damaged cells, only to be obstructed farther on in the bile capillaries. It has been suggested that the small thrombi so often present in the biliary channels are analagous to the casts observed in nephritis.

In pure hemolytic jaundice on the other hand, that type of bile which gives a prompt reaction is not found in the blood. Here icterus follows hemolysis without further action on the part of the liver cells. Much discussion has arisen as to where the second variety of bilirubin is formed. Probably the most logical explanation is that this function is performed by the cells of the reticuloendothelial system. It will be recalled that the endothelial cells lining the blood capillaries of the liver appear somewhat differentiated and react to special staining methods, (the so-called Kupffer cells). They are continuous with other vascular endothelial cells and are quite similar in their general characteristics to the specially differentiated endothelial cells of the spleen. Together with similar cells located elsewhere in the vascular system, this differentiated endothelium forms the reticuloendothelial system. It seems probable that these cells, particularly those in the spleen and liver, deal with the breaking down of hemoglobin and the elaboration of the bile pigment. During experimental blood destruction bile pigment, hemosiderin and fragmented red corpuscles have been observed in the cells of the reticuloendothelial system. That the spleen is of great importance in the production of bile pigment is indicated in the clinical cure of hemolytic jaundice after splenectomy.

The spleen of the goose is very small, while the liver is unusually large. In this animal the cells of the reticuloendothelial system are concentrated in the liver and few are to be found in the spleen. After poisoning with arseniuretted hydrogen the Kuppfer cells of goose liver undergo marked increase in functional activity and proliferate to such an extent that some even become detached and circulate freely in the blood stream. Disintegrated red cells, hemosiderin and bilirubin may be seen within them. Jaundice results from this experiment, but after extirpation of the liver jaundice no longer appears. The suggested explanation is that the greater portion of the reticuloendothelial system has been removed with the liver, with the result that little bilirubin can be formed.

In the rat the spleen is large and highly developed. Following splenectomy in this animal the Kupffer cells of the liver proliferate, apparently to carry on the function of the splenic endothelium. If after splenectomy the Kupffer cells are put out of action by intravenous administration of collargol, experimental icterus is either prevented or greatly delayed. This again indicates that the site of primary bilirubin formation is in the cells of the

reticuloendothelial system. It would appear that in birds, such as the goose, what corresponds to the spleen in higher animals is largely enclosed within the liver.

According to this theory of jaundice the polygonal glandular cells of the liver would appear to be but little concerned in the mechanism of hemolytic jaundice. They have little to do with the manufacture of bile pigment, their function being to transfer it from the vascular capillaries into the bile passages. It seems probable that during this passage the primary bilirubin, giving delayed reaction to van den Bergh's test, becomes modified into a secondary form, giving a prompt or immediate reaction. According to this hypothesis, if bilirubin in the blood gives a prompt reaction, it has passed through the liver cells and has for some reason been resorbed.

With the delicate tests now in use it has been shown that although small amounts of bilirubin are present in normal serum, with increased blood destruction the cells of the reticuloendothelial system must proliferate to handle the disintegrating corpuscles. In man this proliferation is most extensive in the cells of the spleen. This would account for the splenomegaly characteristically observed in hemolytic diseases.

Van den Bergh has also observed a moderate increase of bilirubin in the serum of some apparently healthy individuals of sallow complexion. This is often familial. In these individuals the spleen is not enlarged, the urine contains neither bile nor urobilin and the fragility of the erythrocytes is not increased. Occasionally the spleen is slightly enlarged and urobilin is found in the urine. It has been shown that latent jaundice may exist, with increased bilirubin content of the blood, but of insufficient amount to cause coloration of the skin or mucous membranes or to cause bile pigment to appear in the urine. Such is the case in pernicious anemia, bothriocephalus infection, hepatic cirrhosis, etc. The development of jaundice in the course of salvarsan therapy may be anticipated by the recognition of latent icterus. In fact latent jaundice appears to be a fairly common occurrence in disease. Early in obstructive jaundice, before there has been opportunity for pronounced discoloration of the skin and mucous membranes, the pigment content of the blood may be high, while during recovery, with the tissues still deeply pigmented, the blood content may be quite low. In cases of cholelithiasis without icterus the bilirubin content of the serum appears normal.

Various observers have described a dissociated jaundice in which the bile pigments are retained while the bile salts are excreted through the liver, or vice versa. This would account for the occurrence of pruritus days or weeks before the onset of icterus. The bile salts appear to pass normally through the liver into the intestines in the late stages of catarrhal jaundice. On the other hand the retention of bile salts and normal excretion of pigment has been observed in some cases of hepatic cirrhosis. Dissociated jaundice has not been thoroughly investigated as regards examination of the blood serum. Most observations have been based upon the presence or absence of bile salts and pigment in the urine and feces.

The interpretation of icterus as outlined above appears quite simple.

As with most hypotheses built upon existing knowledge at the time of their formulation, it may not be long before the interpretation must be changed. Too often work which originally appeared unquestionable is shown by later studies to be fallacious. Thus the theory that hemoglobin formation is a function of the reticuloendothelial system, and that cells in various portions of the body may play a part in this activity, is based to a certain extent upon the study by Whipple and Whooper of the "head-thorax circulation." They excluded from the circulation all tissues below the diaphragm and found that hemoglobin injected into the jugular vein was transformed into bilirubin. They concluded that the liver and spleen are not essential for bilirubin formation and that portions of the reticuloendothelial system above the diaphragm may perform this function.

Rich, who has recently checked this work, shows that the liver was not entirely excluded from the head-thorax circulation, anastomoses with the lower intercostal vessels providing a sufficient blood supply to that organ. After complete abdominal evisceration, with removal of both spleen and liver, Rich found that no bilirubin was produced within the head-thorax circulation. He concludes that the liver is necessary for the transformation of circulating hemoglobin into bilirubin. It still remains possible that the Kupffer cells are the site of this change. The evidence so far, however, is entirely inferential.

Thus, while we realize that the hypothesis under consideration may be fallacious, it serves its present purpose, first in clarifying the clinical problem and second, as a new point of departure for further study of the pathologic physiology of jaundice.

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—*W. T. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Outlines of Medical Zoology**

A BOOK written primarily with the idea of furnishing a textbook for the use of public health officers and students of tropical medicine, "Outlines of Medical Zoology" is the outgrowth of a bulletin published in 1921 entitled "Diagnosis of Protozoa and Worm Parasites in Man." The book is divided into three sections, devoted respectively to parasitic protozoa, worms and arthropods. Its chief value lies in its usefulness as a reference book for differential diagnosis.

Environment and Resistance in Tuberculosis†

DR. KRAUSE'S understanding of environment as applied to disease should hold in many conditions other than tuberculosis. Environment means not only one's physical surroundings but also his hygienic environment, the food he eats, the air he breathes and even the mental atmosphere in which he lives. How important, in tuberculosis particularly, is the patient's mental outlook upon life and his attitude towards illness, and how important to him is the degree of optimism among his associates. Krause, in fact, classifies nearly all noninherited factors influencing tuberculosis as environmental. Thus trauma, infection, etc., may be considered as environmental factors predisposing to, or influencing the course of tuberculosis.

A striking thought presented in his essay on resistance is that the general physical condition has often far less to do with the degree of resistance than has the anatomic position of the individual tubercle. A tuberculous focus well out in the parenchyma of the lung is less dangerous than a single tubercle which is ulcerating through into a blood vessel, where it may cause profuse hemorrhage or may discharge infection to all parts of the body.

The literary style is unusually easy and the volume should be of interest both to patient and physician.

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*Outlines of Medical Zoology. With special reference to laboratory and field diagnosis. By Robert W. Hegner, Professor of Protozoology; William W. Cort, Associate Professor of Helminthology, and Francis M. Root, Associate in Medical Entomology. Department of Medical Zoology School of Hygiene and Public Health The Johns Hopkins University. Cloth. Price $2.40. Pp. 175. New York. The Macmillan Company. 1923.

†Environment and Resistance in Tuberculosis. By Allen K. Krause, A.M., M.D., Associate Professor in Medicine, Johns Hopkins University; Director Dows Tuberculosis Research Fund, Johns Hopkins University; Physician in Charge Phipps Tuberculosis, Dispensary Johns Hopkins University; Lecturer Trudeau School of Tuberculosis. Editor American Review of Tuberculosis. Cloth. Price $1.50. Pp. 137. Baltimore. Williams & Wilkins Co. 1923.

*Analytical Microscopy**

A BRIEF text and reference book for use in the microscopic analysis of foods, drugs, etc. The illustrations are plentiful and unusually well done. They add considerably to the value of the book as a reference work and laboratory guide.

Practical Bacteriology, Blood Work and Animal Parasitology†

MAINTAINING its position as a standard textbook and laboratory guide, this work has increased in size and scope throughout its seven editions so that it is now about twice the original volume. One of the chief advantages of this book lies in the readiness with which classifications may be followed. Thus there are many tables for the identification of bacteria and other parasites. The newer laboratory methods for the serologic diagnosis of syphilis are incorporated, including the various flocculation reactions and Kolmer's modification of the Wassermann test. The phenomenon of bacteriophage is dismissed in one paragraph. Probably in the present state of our knowledge this is all that it deserves.

Part Four is an innovation in that it discusses in a differential way the diagnosis of infection in the various regions, organs, tissues and secretions of the body. Such subdivisions are infections of the ocular region, of the nasal and aural cavities, the mouth, pharynx and teeth, focal infection, cutaneous infection, duodenal contents, etc. The author continues to give perhaps greater prominence than it deserves to Ehrlich's graphic method of presenting the side chain theory. The appendix contains many miscellaneous facts and methods of value in clinical laboratory work.

The Microscope‡

A SMALL volume describing the mechanism and physics of the microscope together with its use, and the use of certain microscope accessories.

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*Analytical Microscopy. Its Aims and Methods. By T. Wallis, B.Sc. (London). F.I.C. Ph.C., Member of the Society of Public Analysts, Lecturer in Botany to the Pharmaceutical Society of Great Britain. Cloth. Pp. 149. London. Edward Arnold & Co. 1923.

†Practical Bacteriology, Blood Work and Animal Parasitology. By E. R. Stitt, A.B., Ph.G., Sc.D., LL.D., Rear Admiral, Medical Corps, and Surgeon General, U. S. Navy; Graduate, London School of Tropical Medicine; Member National Board of Medical Examiners; Member Federal Board of Hospitalization; Member Advisory Board, Hygienic Laboratory; Formerly; Commanding Officer and Head of Department of Tropical Medicine, U. S. Naval Medical School; Professor of Tropical Medicine, Georgetown University; Professor of Tropical Medicine, George Washington University; Lecturer in Tropical Medicine, Jefferson Medical College; Associate Professor of Medical Zoology, University of the Philippines. Seventh edition. Revised and enlarged, with 1 plate and 202 other illustrations containing 675 figures. Cloth. Price $5.00. Pp. 766. Philadelphia. P. Blakiston's Son and Co. 1923.

‡The Microscope. By Conrad Beck. Pp. 144. Price, Two shillings and sixpence. R. & J. Beck, Ltd. London, 1923.

*Practical Medicine Series. Vol. I. General Medicine**

DURING the last few years a tendency has developed among medical publishers to issue annual or periodic volumes in which the current medical literature has been abstracted and classified. This furnishes the busy practitioner with a birdseye view of the newer work in various lines. Sometimes it has been well done, but more often the work has been relegated to individuals who have had little medical experience. The volumes then are found to have little practical value. It is a matter of experience that the best works of this type are those in which the reviewing has been done under the supervision of men whose past work renders them authorities in the subjects discussed. The Practical Medicine Series has been in existence for several years and furnishes just this type of information. It has the added value of critical discussion by editors of wide experience. The present volume is unusually complete and fulfils its mission in a very satisfactory way.

Endocrine Diseases†

THE wealth of clinical material which the author has had at his disposal has enabled him to most successfully apply his thorough knowledge of pathologic chemistry to bedside observation and the experimental study of internal secretory disorders. He has summarized his experiences and conclusions in this abundantly illustrated volume. Rather verbose in the original it becomes more so in the English translation, which is rather too literal for easy reading. A disadvantage of most translations and one which is particularly noticeable in that under consideration, is that the editor in order not to alter the author's original text, brings the new edition to date by parenthetical insertions. In this way the continuity of the discussion is sometimes broken. The bibliography which appears at the end of the book is very comprehensive, but it appears to be the original bibliography and contains no reference to the literature of the last few years. These latter references have been incorporated by the editor as foot notes and at the ends of the chapters.

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*Practical Medicine Series, Vol. 1. General Med'cine. Comprising eight volumes on the Year's Progress in Medicine and Surgery. Under the general editorial charge of Charles L. Mix, A.M., M.D., Edited by George W. Weaver, M.D.; Lawrason Brown, M.D.; Robert B. Preble, A.M., M.D.; Bertram W. Sippy, M.D.; Ralph C. Brown, B.S., M.D. Cloth. Pp. 678. Chicago, The Year Book Publishers, 1923.

†Endocrine Diseases. Including their diagnosis and treatment. By Wilhelm Falta, Vienna. Translated and edited by Milton K. Myers, M.D., Neurologist to the Northern Liberties Hospital; to the Lucien Moss Home, Jewish Hospital; to the Dispensary of the St. Agnes Hospital, Philadelphia. With a foreword by Sir. Archibald E. Garrod, K.C.M.G., M.D., (Oxon), F.R., C.P. (London). Third edition, with supplementary notes by the editor 104 illustrations. Cloth. Price $8.50. Pp. 669. Philadelphia. P. Blakiston & Son. 1923.